12-1-1996

Determination of Chromatic and Luminance Channels in the Zebrafish Visual System Using an Increment Threshold Technique

Richard Hughes
Western Kentucky University

DETERMINATION OF CHROMATIC AND LUMINANCE CHANNELS IN THE ZEBRAFISH VISUAL SYSTEM USING AN INCREMENT THRESHOLD TECHNIQUE

A Thesis

Presented to the Faculty of the Department of Psychology

Western Kentucky University

Bowling Green, Kentucky

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

by

Richard Alan Hughes

December 1996

DETERMINATION OF CHROMATIC AND LUMINANCE CHANNELS IN THE ZEBRAFISH VISUAL SYSTEM USING AN INCREMENT THRESHOLD TECHNIQUE

Date Recommended July 31, 1996

Joseph Bilotta
Director of Thesis

Elizabeth Lemerise

Daniel L Roe

Elmer Gray 11-12-96
Dean, Graduate Studies and Research Date

Acknowledgments

Writing this page means that I have been able to accomplish a very difficult, yet rewarding task. Many people have made some contribution to the final product in the pages that follow, and without the support of these persons, this project would not be of the quality which I believe it is. First of all, Dr. Joseph Bilotta played a significant role in all aspects of this project, from the initial ideas, to building equipment, to reading countless drafts. He has helped me to become a better scientist; Dr. Bilotta has effortlessly tried to instill in me his philosophy of science and I believe he has succeeded. Without his patience, knowledge, skill, friendship, and a remarkable ability to teach me the many things I needed to know, completing the project would not have been possible. I would also like to thank Drs. Dan Roenker and Elizabeth Lemerise. They both have given extremely helpful suggestions for improving the quality of this project. I owe many thanks to Warren Patterson. He has helped me more than he probably realizes. He has become a great colleague and wonderful friend. I would like to say a special thanks to my family: my mother, Gloria, my father, Eddie, and my grandparents, Floyd and Irene Lawson. Each of you have been great in supporting my graduate education thus far and I know you will continue to do so. Finally, to Jennifer Sawyers, I must say that I owe much to you for your love and understanding. You have been extremely supportive throughout all aspects of this project.

This project was supported by Kentucky NSF/EPSCOR grant 4-28851-95-208. In addition, Western Kentucky University President's Special Grant Fund and a graduate student research grant provided funding. I would like to extend a special thanks to Drs. Karlene Ball and Paul J. DeMarco, Jr. for the loans of equipment.

Table of Contents

List of Tables

List of Illustrations

DETERMINATION OF CHROMATIC AND LUMINANCE CHANNELS IN THE ZEBRAFISH VISUAL SYSTEM USING AN INCREMENT THRESHOLD TECHNIQUE

Richard Alan Hughes December 1996 77 pages

Directed by: Joseph Bilotta, Ph.D., Elizabeth Lemerise, Ph.D., and Dan Roenker, Ph.D.

Department of Psychology Western Kentucky University

It is well established that the primate visual system possesses anatomically separate chromatic and luminance channels; these pathways are responsible for the visual coding of color and brightness information, respectively. In lower vertebrates, such as the zebrafish (*Danio rerio*), there does not appear to be any anatomical separation of cell types mediating color and luminance information. However, several studies suggest that these vertebrates may be able to discriminate these attributes of a visual stimulus. The purpose of this study was to determine whether the zebrafish visual system possesses chromatic and luminance channels. In addition, the contribution of each cone photoreceptor type to the chromatic and luminance channels was determined. An increment threshold procedure was used to elicit electroretinogram (ERG) b-wave responses to monochromatic light under two different intensities of white background adaptation. From the ERG b-wave responses, a spectral sensitivity function was derived under low and high levels of white background. By examining spectral sensitivity functions under these two levels of white background adaptation, it was possible to determine if two functional channels exist for color and luminance in the zebrafish visual system. The results of this study suggest that zebrafish possess a chromatic channel, but do not seem to have a luminance channel. The low level of white background adaptation yielded a spectral sensitivity function that was similar to the spectral sensitivity function of the chromatic channel in the primate. Alternatively, the high

level of white background produced a spectral sensitivity function which appears to represent neither a chromatic nor a luminance channel; rather, the high level of white background only suppressed the cone contributions to the spectral sensitivity function. In addition, the ultraviolet cones were found to contribute substantially to spectral sensitivity functions derived under both the high and low white background adaptation levels. In conclusion, the results of the present study suggest that anatomical separation may be required for separation of function. Furthermore, the finding that zebrafish possess only a chromatic channel, as well as substantial ultraviolet sensitivity, may be a reflection of the type of environment in which they are normally found.

Chapter 1

Introduction and Purpose of This Study

Our experience of the world comes from a variety of sensory modalities. However, of all the ways humans are provided with information about the environment, vision is perhaps the one relied on most. Because of its importance to our daily lives, it is imperative to determine how form, color, brightness, and motion are represented in the nervous system, and how these attributes are brought together to produce visual perception.

Within the past two decades, a significant amount of research has been directed at understanding how different aspects of a visual scene are processed by the visual system. These studies have shown that primate visual systems possess several separate anatomical pathways or channels. The neurons in these pathways have distinct structural and physiological characteristics which suggest that each pathway is designed to transmit certain aspects of a visual stimulus separately. Two pathways in particular, the chromatic and luminance channels (often referred to as the color-opponent and broad-band channels) have received much attention because it is believed these pathways are responsible for the visual coding of color and brightness information, respectively. A number of studies (see Livingstone & Hubel, 1987 for a review) have established that the chromatic channel, in addition to signaling color information, mediates visual information related to acuity and form perception. Alternatively, it has been shown that the luminance channel, in addition to signaling brightness information, provides information about contrast and motion. Thus in higher vertebrates (including humans), visual perception is mediated by separate anatomical pathways which are initiated in the retina and remain separate through higher

visual centers in the brain.

Although the visual systems of higher vertebrates appear to have functionally separate visual channels, there are a number of studies that demonstrate that some properties of the chromatic channel are mimicked by the luminance channel (Shapley, 1990). This "double-duty" may be an important characteristic of vision in lower vertebrates. Many lower vertebrates are capable of color perception; it appears that, at least physiologically, they process color in the same fashion as higher vertebrates. However, in some lower vertebrates, such as fish, there does not appear to be anatomical segregation of cell types mediating color and luminance information. This raises the issue of whether the processing of color and luminance information along separate channels is a fundamental component of all visual systems capable of color perception.

The literature review which follows is divided into four main sections. The first section describes the basic anatomy and physiology of the vertebrate retina. In the second section, the concept of the spectral sensitivity function and how it can be used as a tool to determine a species' ability to encode different wavelengths of light are discussed. The third section provides an overview of research related to parallel visual channels, including evidence for separate processing of color and luminance information in lower vertebrates. Finally, the fourth section introduces the zebrafish (*Danio rerio*) as a model for vision research. The literature review concludes by discussing the purpose of this thesis and outlines the specific issues to be addressed.

Basic Anatomy and Physiology of the Retina

The retinas of most vertebrates contain five classes of neurons: the photoreceptors (rods and cones), horizontal, bipolar, amacrine, and ganglion cells. The retina represents the initial stage of visual processing, which progresses in a somewhat sequential manner through the retina. Visual processing begins in the retina; light energy is converted into neural energy by the photoreceptors. Once a neural signal is initiated in the photoreceptors,

the signal is sent to horizontal cells and bipolar cells. From these cells, the neural signal is sent to amacrine cells. Finally, the signal is sent to ganglion cells (Levine & Shefner, 1981). In vertebrates, the ganglion cell axons comprise the optic nerve; the optic nerve carries visual information to higher visual centers in the brain.

An interesting property of visual processing is that neurons beyond the photoreceptors demonstrate different response patterns to illumination. Different response patterns to illumination is first observed in bipolar cells, and remains a visual property through higher visual centers. This response behavior is the result of a neuron's receptive field, which is a region of the retina, that when illuminated influences the response pattern of that neuron. Much of what is known about receptive field properties has come from single unit recordings of ganglion cells. For example, recording from cat ganglion cells, Kuffler (1953) first discovered that the response patterns of ganglion cells were a function of the precise location in which incident light was projected onto its receptive field. When spots of light were presented to different parts of a ganglion cell's receptive field, the cell's response changed. For instance, for some cells, light focused on the center area of the receptive field caused the activity of the cell to be greater (i.e., excitation) than its spontaneous rate (the spontaneous rate is the firing rate of a neuron in the absence of visual stimulation). However, light incident on the surround area of the receptive field produced a decrease (i.e., inhibition) in its response relative to the spontaneous rate. These cells were classified as ON-center cells. In addition, ganglion cells with the opposite receptive field organization were found in Kuffler's study. These cells responded with a decline of activity when light was projected to the center and the response increased when light was incident on the surround. These cells were termed OFF-center cells.

The early work of Kuffler showed that retinal ganglion cells respond differently to patterns of illumination and suggests that neurons in the vertebrate visual system are specialized to encode different types of visual stimuli.

Spectral Sensitivity

Although many vertebrates share basic retinal anatomy and physiology, vertebrate species vary in their ability to respond to different wavelengths of light. This difference is illustrated in what is known as the spectral sensitivity function. Spectral sensitivity functions depict the sensitivity of a visual system to different wavelengths of light. The differences in spectral sensitivity observed across species are due to several factors. First, the number of different cone types is fundamental to the spectral sensitivity of a visual system (Zrenner, Abramov, Akita, Cowey, Livingstone, & Valberg, 1990) and dictates the spectral range to which a visual system is responsive. The sensitivity of a single cone photopigment to light is reflected in its cone absorption spectra, which is how much light energy the photopigment absorbs across a restricted region of the spectrum. The wavelength to which a photopigment is maximally absorpant is referred to as its lambda-max (λ-max). As wavelength is increased or decreased relative to the λ-max, less light energy is absorbed by the photopigment. Wavelengths that do not fall within a photopigment's spectral range are not absorbed; thus the cone is unresponsive to those wavelengths.

The most basic requirements for color perception demand that a system possess at least two cone types, each having a different photopigment, and that the cone types have overlapping spectral sensitivities (Cornsweet, 1977). Higher vertebrates such as humans are considered trichromatic since they have three cone types. Each cone type possesses a photopigment which is maximally sensitive to light from either the short (S-cones), middle (M-cones), or long (L-cones) wavelength region of the visible spectrum (Sperling &

Harwerth, 1971; Zrenner et al., 1990). However, other vertebrate retinas, such as tree shrew (Petry, 1993), contain cones sensitive only to middle and long wavelengths of light. Furthermore, some vertebrates, in addition to having cones sensitive to short, medium, and long wavelengths of light, possess cone types capable of detecting ultraviolet light (Goldsmith, 1994). Sensitivity to ultraviolet light has been demonstrated in a number of birds (Goldsmith, 1994), rodents (Jacobs & Deegan, 1994), and in some fish (Hawryshyn, Chou, & Beauchamp, 1985) including carp (Hawryshyn & Harosi, 1991), rainbow trout (Hawryshyn & Harosi, 1994), goldfish (Hawryshyn & Beauchamp, 1985; Neumeyer, 1985), and the zebrafish (Robinson, Schmitt, Harosi, Reece, & Dowling, 1993).

A popular method for determining spectral sensitivity is referred to as increment threshold. The increment threshold procedure consists of superimposing monochromatic light onto a white background and varying the intensity of the monochromatic stimulus until a predetermined response criterion is reached. Sensitivity is defined as the inverse of the log intensity needed to reach a criterion response at each stimulus wavelength. From this information, a spectral sensitivity function can be produced which plots log sensitivity as a function of stimulus wavelength. Increment threshold spectral sensitivity has been determined using psychophysical methods in humans (King-Smith & Carden, 1976), primates (Sperling & Harwerth, 1971), fish (Neumeyer, 1984), cats (Loop, Millican, & Thomas, 1987), tree shrews (Petry, 1993), and rodents (Jacobs & Deegan, 1994). In addition, the increment threshold technique has been used to derive spectral sensitivity functions from electrophysiological responses (e.g., Mills & Sperling, 1990).

The spectral sensitivity function is a valuable tool because it provides a way to determine the contributions of the different cone types to visual processing. Traditionally, two methods have been used to determine the cone contributions to the spectral sensitivity function. One procedure involves visually comparing spectral sensitivity functions to the

known absorption spectra of each cone type. In this procedure, templates of the cone absorption spectra are superimposed over the obtained spectral sensitivity function (see Mackintosh, Bilotta, & Abramov, 1987); although this technique allows one to determine qualitatively the cone contributions to the spectral sensitivity function, the precise contribution of each cone type is not discernible from this method. Alternatively, computational modeling has been used to determine cone contributions from spectral sensitivity functions (DeMarco & Powers, 1991; Coughlin & Hawryshyn, 1994a, 1994b). This procedure uses an equation to derive the relative weight each cone type contributes to the overall spectral sensitivity function. Computational modeling permits a more quantitative analysis of cone contribution than does the template method.

In sum, the spectral sensitivity function is useful in determining which cone types contribute to visually mediated behavior. Additionally, spectral sensitivity functions across various conditions have been used to suggest that there are separate functional visual channels and that each channel serves a different role in visual function. Therefore the spectral sensitivity function can be used to serve two purposes: It provides a means to verify that there are different functional channels in a visual system, and to determine what contribution, if any, different cone types make to a visual channel.

Parallel Processing

Anatomical evidence. Research with primates and other higher vertebrates shows that anatomically separate pathways are formed in the mammalian retina and remain segregated through higher visual centers (Schiller & Malpeli, 1978; Lennie, 1980; Schiller, Logothetis, & Charles, 1990; Livingstone & Hubel, 1987, 1988; Merigan & Maunsell, 1990). In higher vertebrates, axons of retinal ganglion cells selectively project to either the magnocellular or parvocellular layers of the lateral geniculate nucleus (LGN) of the thalamus. Retinal ganglion cells which project to the parvocellular layers of the LGN are

referred to as P-cells; likewise, retinal ganglion cells which project to the magnocellular layers of the LGN are called M-cells. Furthermore, even upon leaving the LGN, there remains anatomical separation of these pathways to the higher visual centers in the cortex (Schiller & Malpeli, 1978; Livingstone & Hubel, 1987, 1988). The role each pathway plays in visual function is discussed below.

Physiological evidence. The first physiological evidence for separate visual channels came from the work of DeValois, Abramov, and Jacobs (1966). DeValois et al. obtained responses of recordings from 147 LGN cells, and classified these cells according to response characteristics to presentations of different wavelengths of light. Based on their responses, DeValois et al. were able to classify LGN cells in two broad categories: Spectrally nonopponent and spectrally opponent cells. Spectrally nonopponent cells demonstrated the same type of response (i.e., excitation) to all wavelengths of light. It is important to note that although spectrally nonopponent cells responded the same to all wavelengths of light, some wavelengths produced more excitation than others. In contrast, spectrally opponent cells demonstrated response patterns that were dependent upon the wavelength of light. These cells responded with excitation to some wavelengths and with inhibition to others. Four types of opponent cells were found. One type of cell was found to show excitation to red light and inhibition to green light. These cells were classified as +R-G cells. Cells with the reverse responses were found as well. These cells responded with excitation to green light and inhibition to red light and were called (+G-R). A third cell type was found which was excited by yellow light and inhibited by blue, and was classified as a +Y-B cell. Finally, a group of cells was found which was excited by blue light and inhibited by yellow (+B-Y).

More recent studies have found that the color opponency observed in some LGN neurons is initiated in primate ganglion cells (Gouras & Zrenner, 1981; Livingstone & Hubel, 1987, 1988). Although spectral opponency is first observed in bipolar cells, there

has been a greater interest in spectral opponency at the ganglion cell layer. P-cells exhibit color opponency which is spatially organized in a center and surround fashion (Gouras & Zrenner, 1981; Zrenner et al., 1990). While a stimulus of a particular wavelength incident on the center mechanism will increase activity of a P-cell, surround illumination of a different wavelength will inhibit cell activity. Comparatively, M-cells in general do not demonstrate color opponency and respond with the same type of response to all wavelengths of light. It has been shown, however, that some M-cells in the primate retina do show spectral opponency (see Shapley, 1990 for a review).

The neural basis for whether a cell is spectrally opponent or nonopponent is related to how signals from the different cone types converge onto spectrally opponent P-cells and nonopponent M-cells (Zrenner et al. , 1990). It has been suggested that in human and the retinas of other higher vertebrates, the M- and L-cones contribute to responses of M-cells (Livingstone & Hubel, 1987; Eisner & Macleod, 1979); thus spectrally nonopponent M-cells are driven by additive cone responses in which signals produced by the M- and L-cones are added together. Since M- and L-cone responses are simply added together, M-cells lose their ability to code for wavelength because there is no opponency, which is a requirement for color vision (Sekuler & Blake, 1994). Spectral sensitivity functions for M-cells have been found to be smooth and broad-band and peak around 535 nm (Zrenner et al., 1990).

For spectrally opponent P-cells, responses are mediated by the addition and subtraction of cone signals. Thus, the responses of color opponent ganglion cells depend on whether an excitatory or inhibitory synapse exists somewhere between a cone type and the ganglion cell. Two different classes of P-cells are distinguished based on whether they show red/green opponency or blue/yellow opponency. This distinction reflects that P-cells demonstrating red/green opponency receive different cone inputs than ganglion cells that

show yellow/blue opponency (Zrenner et al. 1990). Cones which converge on P-cells showing red/green opponency signal the difference between stimulation of the M-cones and L-cones. In contrast, cones which converge onto P-cells showing blue/yellow opponency signal the difference between the S-cone signals and the summation of M- and L-cone signals (Sekuler & Blake, 1994). The spectral opponency observed in P-cells is reflected in their spectral sensitivity function, which shows several notches usually between the λ-max of the contributing cone types.

Since spectrally opponent P-cells and spectrally nonopponent M-cells originate in the retina and remain segregated through higher visual centers, these pathways have been thought of as distinct channels which process color and luminance information as separate phenomena (Merigan, 1989; Merigan & Maunsell, 1993; Shapley, 1990; Livingstone & Hubel, 1987). In addition to separately processing color and luminance information, recent studies suggest that the chromatic and luminance channels in primates are designed to visually encode other types of visual information (Merigan, 1989; Schiller & Logothetis, 1990; Schiller et al. 1990; Schiller, Logothetis, & Charles, 1991; Shapley, 1990). Extensive anatomical and physiological studies on the primate retina indicate that neurons which comprise the chromatic and luminance channels have distinct structural and neural properties which suggests these pathways play different roles in visual function (Gouras & Zrenner, 1981). Studies show that M-cells are larger, conduct information much faster, respond well to temporal stimuli, and have very large receptive fields. In contrast, P-cells are smaller, conduct information slower, do not respond well to temporal information, and have small receptive fields. Also, the population density of the two cell types differ. Approximately 90 percent of all ganglion cells in primate retinas are P-cells (Livingstone & Hubel, 1987).

Based on these properties specific to M-and P-cells, a number of studies have shown that these cell types represent the luminance and chromatic channels, respectively

(Lennie, 1980; Schiller et al., 1990; Livingstone & Hubel, 1987, 1988; Merigan, 1989; Schiller & Logothetis, 1990). For instance, Schiller et al. (1990) investigated the functioning of chromatic and luminance channels by inducing selective lesions to either the magnocellular or parvocellular region of the LGN. They found that parvocellular lesions impaired color vision, high spatial frequency form vision, and fine stereopsis. Comparatively, lesions to the magnocellular region produced deficits in high temporal frequency and motion perception. However, low spatial frequency form perception, stereopsis, and brightness perception were not affected with either lesion. The results of this study suggested that the chromatic channel mediates wavelength and spatial information, whereas the luminance channel conveys information about the temporal properties of a stimulus.

Psychophysical evidence. In addition to anatomical and physiological documentation for parallel visual channels in higher vertebrates, several studies provide psychophysical evidence for the existence of functionally separate visual channels. In human psychophysics, it has been shown that a channel exists for the processing of chromatic information, and another which processes luminance information (Sekuler & Blake, 1994). King-Smith and Carden (1976) provided a detailed analysis of the spectral characteristics of the luminance and chromatic channels and showed that each might serve functionally different roles in visual perception. The purpose of their study was to determine why under some circumstances the contributions of the luminance system to visual detection had not been observed behaviorally, although it had been demonstrated physiologically by DeValois et al. (1966). They found that both luminance and chromatic channels contribute to visual perception, although each have different thresholds; thus, for a stimulus to be processed by a channel, it must first exceed the threshold for that channel. When subjects were presented with small, brief flashes of light, the spectral sensitivity

functions were smooth and broad-band, showing no indication of opponent mechanisms. This observation was evidence for the existence of a luminance channel. However, if longer and much larger test stimuli were used, spectral sensitivity functions exhibited a decline in sensitivity at some wavelengths and an increase in sensitivity at others; these depressions in sensitivity or "notches" are the result of opponent mechanisms (see below). Spectral sensitivity functions derived under these conditions provided evidence for the existence of the chromatic channel. King-Smith and Carden (1976) showed that the chromatic and luminance channels could be isolated simply by changing stimulus parameters. They were able to demonstrate psychophyisically that human vision could be represented by chromatic and luminance channels, and that each system has a different threshold of activation.

Petry (1993; see also Petry & Kelly, 1991) provides recent psychophysical evidence [in the tree shrew] of separate channels for luminance and chromatic processing. Petry determined threshold under three different levels of light adaptation; depending on the intensity of adaptation, different spectral sensitivity functions were found. Behavioral results showed that the spectral sensitivity functions under high levels of adaptation showed a decline in sensitivity at some wavelengths, or notches in the spectral sensitivity function; however, spectral sensitivity under low levels of light adaptation was smooth and broad-band. Petry showed that chromatic and luminance channels could be isolated behaviorally in tree shrews, depending upon the physical characteristics of the test stimulus and the intensity of background adaptation.

The above studies indicate that different aspects of a visual stimulus are processed along anatomically and functionally separate channels in higher vertebrates. In these vertebrates, neurons in the retina selectively project along separate tracts to different regions of the LGN and cortex, and each channel processes specific attributes of a visual scene. Many lower vertebrates such as fish do not possess these anatomically separate pathways

or channels. However, the basic retinal anatomy of these and higher vertebrates is similar in that each possess the necessary equipment for color perception. Furthermore, color vision has been demonstrated behaviorally in some fish species, including goldfish (Yager, 1967). Although there is no anatomical evidence for functionally separate visual channels in fish as found in higher vertebrates, there is some compelling physiological and behavioral evidence for separate processing. However, the mechanisms which enable the visual systems of fish to discriminate different qualities of a visual stimulus are unclear.

Parallel Processing in Lower Vertebrates

There is some evidence which suggests that separate processing of color and luminance information may exist in lower vertebrates such as fish. Physiological evidence has come from single unit recording studies, in which there has been documentation of spectrally opponent and nonopponent cells in the retina and other regions of the visual system. Coughlin and Hawryshyn (1994a) studied the spectral sensitivity of cells in the optic nerve and optic tectum of rainbow trout to determine the contribution of ultraviolet cones to the spectral sensitivity functions. In lower vertebrates such as fish, the optic tectum receives information from the retina, and is functionally similar to the visual cortex in primates. This study showed that both spectrally opponent and spectrally nonopponent cells were present in both the optic nerve and optic tectum. Mackintosh, Bilotta, and Abramov (1987) also showed that spectrally opponent and nonopponent ganglion cells are present in goldfish retina. Mackintosh et al. were interested in determining the contribution of S-cones to goldfish ganglion cells. In addition to determining S-cone contribution to ganglion cells, ganglion cells were classified as spectrally opponent and nonopponent. The Macintosh et al. study also suggests that a particular ganglion cell might perform a dual function in visual processing. By changing stimulus parameters through chromatic adaptation, they found that a nonopponent ganglion cell could perform the function of an

opponent ganglion cell. The Coughlin and Hawryshyn and Macintosh et al. studies suggest that separate processing of color and luminance may occur in fish given that spectrally opponent and nonopponent cells are present.

There have been several studies which provide some interesting psychophysical evidence for chromatic and luminance channels in fish. Neumeyer (1984), using an increment threshold procedure with a white background, found that the spectral sensitivity function of the goldfish showed evidence of inhibitory interactions between cone types in the retina. Compared to the cone absorptance spectra for goldfish, the spectral sensitivity data showed narrower peaks in sensitivity; Neumeyer used this finding to suggest the possibility of chromatic inhibition in goldfish. Additionally, Neumeyer, Wietsma and Spekreijse (1991; see also Spekreijse, Wietsma, & Neumeyer, 1991) have demonstrated behaviorally that goldfish might process color and luminance differently. They showed that in wavelength discrimination tasks, goldfish used different criteria (i.e., color or brightness) as a basis for discrimination. However, spectral sensitivity functions were not obtained under conditions that would isolate chromatic and luminance channels. Therefore, this study does not provide a detailed analysis of separate processing of chromatic and luminance information in fish.

Even though fish do not possess separate anatomical pathways for color and luminance, it is still possible that their visual systems can discriminate these phenomena. There have been several studies that offer an explanation as to how a visual system without separate anatomical pathways could process different types of visual information. Gouras and Zrenner (1979) found that the temporal properties of monkey ganglion cells may enable these cells to code for different types of visual information. For instance, they observed that at high temporal frequencies, color-opponent ganglion cells in macaques have broad-band spectral sensitivity functions, whereas at low temporal frequencies, these same cells show chromatic inhibition. The physiological mechanisms for these observations are

related to the center and surround portions of a cell's receptive field. Gouras and Zrenner found that at very low temporal frequencies, the center and surround portions of the receptive field responded antagonistically to one another, which produced spectral sensitivity functions reflective of color opponent processes; however, at high temporal frequencies, center and surround mechanisms became synergistic, enhancing the cell's ability to detect changes in luminance. This synergistic mechanism is due to a frequency-dependent phase shift between the center and surround mechanisms. Gouras and Zrenner showed that at low temporal frequencies, center and surround responses were out-of-phase or antagonistic; however, at high temporal frequencies, center and surround responses were in-phase or synergistic.

Bilotta and Abramov (1989a) obtained similar results studying the spatial properties of goldfish ganglion cells using sinusoidal gratings drifting across a cell's receptive field. One finding from this study was that the cell's spatial filtering ability at different drift rates depended on the interplay between the center and surround mechanisms of the receptive field. For instance, at low temporal frequencies, the center and surround of the receptive field were found to be antagonistic to one another. At low temporal frequencies, ganglion cell center and surround responses were out-of-phase, or mutually antagonistic. However, at high temporal frequencies, center and surround responses were synergistic which made the cell more responsive to a fast moving stimulus. Thus, Gouras and Zrenner (1979), as well as Bilotta and Abramov (1989a), show that the interactions between center and surround mechanisms of a cell's receptive field enable a neuron to transmit different properties of a visual stimulus. Thus, it may be possible for a single cell to have dual abilities which become evident by altering the temporal properties of a visual stimulus. Therefore, lower vertebrates such as fish may process luminance and color information separately, although this information is not anatomically separated in their

visual systems like in higher vertebrates.

This section represents a review of the literature relevant to parallel visual channels in the visual systems of vertebrates. It has been shown that in higher vertebrate visual systems, such as primates and humans, anatomically separate pathways are formed in the retina and remain separate through higher visual centers. A major property which distinguishes cells in these pathways is whether they show spectral opponency. Cells which show spectral opponency encode wavelength information about a stimulus, whereas spectrally nonopponent cells mediate luminance information about a stimulus. These two different cell types, often referred to as chromatic and luminance channels, are specialized to encode other aspects of a visual scene, as well as separately processing wavelength and luminance information. Therefore in higher vertebrates, several different attributes of a stimulus are carried along anatomically and functionally separate neural channels. However, some lower vertebrates such as fish do not have separate anatomical pathways. Nevertheless, a number of studies have provided physiological and behavioral evidence for separate processing of chromatic and luminance information in fish.

The Zebrafish as a Model of Vision

Recently the zebrafish, *Danio rerio*, has sparked an interest in science as a new model for genetics, neuroscience, and developmental biology (Barinaga, 1990). The usefulness of the zebrafish to these disciplines is due to several attributes that make it desirable for scientific study. For instance, the zebrafish is easy to breed and reaches adulthood by three months of age. Also, zebrafish embryos develop in transparent eggs which make it possible to implement environmental manipulations and then examine such effects (e.g., Bilotta, Dobis, Googe, Nunley, & DeLorenzo, 1996).

The zebrafish is of particular interest for vision research for several reasons. First, research has shown that the zebrafish is a very visual animal (Laale, 1977) and has a well developed visual system at adulthood (Branchek, 1984). Second, studies of the

anatomical organization of the zebrafish visual system suggest that this vertebrate shares the basic retinal anatomy found in other vertebrates, including humans. However, there has been some disagreement regarding whether zebrafish possess a fourth ultraviolet cone photopigment. Robinson et al. (1993) found that, like other vertebrates, zebrafish have three cone types which are sensitive to short, middle, and long wavelengths regions of the visible spectrum. These cone types have peak sensitivities of 415, 480, and 570 nanometers (nm). In addition, they found that the zebrafish also possess an ultraviolet receptor that has a peak sensitivity of 360 nm. They reported that the ultraviolet cone type constitutes approximately 25% of the cone population. Furthermore, each type of photopigment found in zebrafish occupies anatomically different cone types. Like other fish, there is a double cone which contains two outer segments with different photopigments. The 570 nm pigment is found in the long outer segment and the 480 nm pigment is contained in the short outer segment of the double cone. There is also a single long cone which holds the 415 nm pigment, and the ultraviolet pigment (360 nm) is found in the short single cones. However, an earlier study (Nawrocki, BreMiller, Streisinger, & Kaplan, 1985) found no evidence for an ultraviolet photopigment in any of the four anatomical cone types in the zebrafish retina. Robinson et al. (1993) argue that the discrepancy between their data and that of Nawrocki et al. is based on methodological limitations of the Nawrocki study to isolate ultraviolet pigments. To date, there are no physiological studies that have investigated color vision, or the physiological contribution of the ultraviolet cones in zebrafish.

Summary

It has been shown that in higher vertebrates the luminance and chromatic channels are designed to transmit certain aspects of a visual scene. These channels appear to maintain a segregation of color, form, motion, luminance, and flicker. In fish, however,

there does not appear to be any anatomical segregation of different cell types mediating color and luminance pathways equivalent to that found in primate retinas. Although anatomical studies have been done on the structure of the zebrafish retina, there is no empirical evidence that retinal neurons project in separate neural channels to higher visual centers. However, given the similarity among the mammalian and the zebrafish retina, and psychophysical and physiological evidence for color and luminance processing in fish, it is plausible that separate processing of color and luminance may be present in zebrafish.

The first issue of this project will address whether the processing of color and luminance information is represented by separate channels in the zebrafish visual system. Since the luminance and chromatic channels appear to operate under different stimulus conditions in higher vertebrates, experimental manipulations will be used to isolate these two channels in zebrafish. To isolate chromatic and luminance responses, spectral sensitivity functions based on electroretinogram (ERG) responses will be derived under two different levels of white background adaptation. The ERG is a non-invasive technique which provides a gross measure of the electrical activity of the retina. It is composed of several components, or waves, and each wave represents the electrical activity of a particular type of retinal neuron. The first wave comprising the ERG is the a-wave. This wave demonstrates a slight negative response that reflects the electrical activity of the photoreceptors. The second component of the ERG is the positive b-wave, which is believed to represent electrical activity of the bipolar cells (see DeMarco & Powers, 1989). The responses of bipolar cells are important since bipolar cells represent the first layer of retinal neurons capable of providing color opponency.

By examining the spectral sensitivity functions under two different white background adaptations, it may be possible to determine if two functional channels exist and which channel is functioning under a particular adaptation level. It is predicted that color and luminance will be represented in two separate channels in the zebrafish. Under

the low white background adaptation, it is predicted that the spectral sensitivity function will be smooth and broad-band, which is indicative of the luminance channel. Under the high level of white background adaptation, it is predicted that the spectral sensitivity function will show several notches and dips which are characteristic of the chromatic channel. Spectral sensitivity functions will provide a means to determine if functionally separate channels exist in zebrafish since spectral sensitivity functions of the luminance and chromatic channels theoretically must differ as they do in higher vertebrates.

The second issue of this project is to determine which of the four cone types found in the zebrafish retina contribute to the spectral sensitivity functions of the chromatic and luminance channels. Based upon work in other species, it is predicted that the spectral sensitivity function under the low white background adaptation in zebrafish will show additive cone responses, with contributions from at least the M-and L-cone types. It also is predicted that the chromatic channel will show a decline in sensitivity at some wavelengths due to color opponency. It also is hypothesized that the ultraviolet cones will contribute to spectral sensitivity of the luminance channel. It has been shown that in the rainbow trout, ultraviolet cones contribute to some cells mediating luminance information, although M- and L-cones make a greater contribution. In the literature, there is some discrepancy about the role ultraviolet receptors play in processing color and luminance information. The investigator will attempt to determine the contribution that ultraviolet receptors make to the chromatic and luminance channels.

Chapter 2

Method

Participants

Thirteen male and female adult zebrafish (*Danio rerio;* Scientific Fisheries, Huntington Beach, CA) measuring 3 to 4 cm in length were maintained on a 14h light on/10h light off cycle. Fish were fed Tetramin basic flake tropical fish food once each day. The use of animals and animal care was in accordance with guidelines approved by the Institutional Animal Care and Use Committee of Western Kentucky University on January 24, 1996.

Apparatus

Electrophysiological preparation. The holding chamber for the fish was housed in a Faraday cage measuring 76.2 cm high X 55.9 cm wide X 45.7 cm deep. Electroretinograms (ERGs) were obtained using a 36 gauge chlorided silver electrode. The electrode was placed into a micromanipulator (WPI, Sarasota, FL, Model MM-3) held in a magnetic base. A second electrode, also a 36 gauge chlorided silver wire held in an adjustable arm with a magnetic base (Stoelting, Chicago, IL, Model 55145), was used for reference. A stereomicroscope (Edmund-Scientific, Barrington, NJ, Model D39,361) was used by the experimenter for positioning the electrodes. The electrical signals from the two electrodes were AC differentially amplified with a band-pass of 0.1 to 100 Hz (WPI, Sarasota, FL, DAM-50). Signals from the amplifier were sent to a 60 MHz dual-channel oscilloscope (Tektronix, Beaverton, OR, Model 2215A) and sent simultaneously via an A/D converter to a data acquisition board (Scientific Solutions, Solon, OH, Lab Master DMA) of the laboratory computer (DTK, Chicago, IL, Tech-1663). The sampling rate for

the A/D converter was 250 Hz

Optical system. Light stimuli were presented to the cornea via a two-channel Maxwellian view optical system. The entire optical system was mounted on an optics breadboard (ArrowTech, Pittsburgh, PA, Model V 2448), measuring 24 X 28 inches. The test channel was used for generating visible, as well as ultraviolet light, with a spectral range of 320 to 700 nm. The light source for the test channel was a 150 Watt xenon arc lamp (Spectral Energy, Westwood, NJ, Model LH 150). Light projected from the arc lamp was collimated through a quartz window (quartz glass transmits ultraviolet light) and entered a water bath which removed infrared light; the light was focused by a quartz lens onto a shutter controlled by a stepper motor (Alpha Products, Fairfield, CT, Model ST-143). The stepper motor was controlled by the laboratory computer. After passing through the shutter, light was recollimated and passed through an interference filter measuring 50 mm in diameter. Interference filters were used to control stimulus wavelength. Filters for the visible light ranged from 400 to 700 nm in 20 nm steps with a half-bandwidth of 10 nm (Oriel, Stratford, CT, Model 54161). There were also four ultraviolet filters (Andover Corporation, Andover, NH, Model FS10-50) with maximum transmission at 320, 340, 360, and 380 nm, also with a half-bandwidth of 10 nm. To control stimulus irradiance, the collimated light then passed through a series of quartz neutral density filters (Reynard, Calle Sombra, CA, Model 390) measuring 50 mm square. By stacking these filters, it was possible to vary stimulus irradiance over a seven log unit range. The maximum log irradiance was designated as 0.0 log units (i.e., no attenuation) and the minimum log irradiance was -7.0 log units. All test stimuli measurements were made using a radiometer (E.G. & G., San Diego, CA, Model 550-2) and measured in nanoamps which were converted to irradiance units (quanta/cm^2/s). Appendix A shows

the irradiance (in quanta/cm^2/s) emitted at 0.0 log units attenuation at each wavelength. Finally, light passed through a polka dot mixing cube (Oriel, Stratford, CT, Model 38106) measuring 51mm square and focused via a lens just in front of a 5 mm diameter liquid light guide (Oriel, Stratford, CT, Model 22556) so that the light filled the guide.

The second channel generated white light used as background adaptation. The light source for the background channel was a 250 Watt tungsten-halogen bulb (Oriel, Stratford, CT, Model 6334) powered by a 24 volt/12 amp DC power supply (Condor, Oxnard, CA, Model F24-12-A+). Light projected from the tungsten bulb passed through a KG-2 heat filter (Roland Optics, Corina, CA, Model 65.3025) measuring 50 mm square. The filtered light was focused by a lens onto a shutter and recollimated through a series of neutral density filters (Reynard, Calle Sombra, CA, Model 390) measuring 50 mm square. Finally, the light was reflected by a mirror onto the polka-dot mixing cube where it was combined and focused with light from the test channel. Thus, the two beams were superimposed onto one another to fill the liquid light guide.

Procedure

Animals were anesthetized by submersion in a 0.04% solution of tricaine methanesulfonate. Once respiration had stopped, animals were paralyzed with a 200 mg intramuscular injection of gallamine triethiodide. A 26 gauge syringe then was used to make a small incision in the sclera of the right eye. Next, the fish was placed in a small plastic holder to secure the animal during the electrophysiological recordings. The holder was placed into a groove cut into the center of a small sponge, and this sponge was placed on the base of a rectangular plexiglas chamber 3.8 cm high X 7.6 cm long X 3.3 cm wide. The holder had a hollow plexiglas tube on its front and back sides which served as water intake and outake used to artificially respire the fish. A small opening measuring 3.0 cm X 2.3 cm was cut from the right side of the chamber to allow a clear path between the light

guide and the fish's right eye.

Fish were artificially respired by continually passing an aerated solution of 0.01% tricaine methanesulfonate through the gills. This procedure ensured that the animal remained anesthetized throughout a testing session. Prior to each session, the holding chamber was filled with water until the water line was just below the opening on the side of the chamber. A water pump (Aquarium Systems, OH, Model MN404) was submerged in a two gallon aquarium located under the table where physiological recordings were done. Connected to this pump was aquarium tubing; this tubing was connected to the intake tip of the holding chamber.

Once the anesthetized fish was placed in the chamber, the experimenter using the stereomicroscope for visual guidance, placed the fish's mouth on the water intake tube. During recordings, the eyes were kept above the water line and moistened using a fish saline (Ringer's) solution. When the fish was positioned in the holding chamber, the electrode was lowered by the micromanipulator into the right eye. After this electrode was in place, the reference electrode was then placed on the fish's nostril. Once both electrodes were positioned correctly, a test session began. To provide optimal stimulus conditions for the luminance channel, a background adaptation of 0.0053 $\mu W/cm^2$ (-6.0 log units attenuation) was used. This background level was low enough to be below threshold for the chromatic channel for humans (King-Smith & Carden, 1976), but greater than the minimum intensity required for cone vision in zebrafish (Branchek, 1984). To isolate the chromatic channel, a background adaptation of 4.36×10^2 $\mu W/cm^2$ (-1.0 log units attenuation) was used. This background level exceeds threshold for the luminance channel in humans (King-Smith & Carden, 1976).

A test session consisted of presenting monochromatic light at various irradiances under the low and high background adaptation levels for one fish. To begin a session, the shutter from the background channel of the optical system was opened to provide the white background adaptation light. Fish were first adapted to one background for 20 minutes. Next, an interference filter was inserted in the path of the test channel. Wavelengths of 320, 340, 360, 380, 400, 420, 440, 480, 520, 560, 600, 640, and 660 nm were used since they centered around wavelengths which correspond either to the λ-maxs of the four cone photopigments or between the l-maxs of two cone photopigments. The starting wavelength was selected from either the short or long wavelength end of the spectrum and the starting order was varied across test sessions. For example, if on one session, 660 nm was chosen as the starting value, then on the next session (which was a different subject), 320 nm served as a starting point. For each session, stimulus wavelength order was staggered to avoid chromatically adapting any one cone type and to ensure that sufficient wavelength information was collected for any one fish. Starting at the initial wavelength, stimulus wavelengths were varied by skipping every other wavelength until the other end of the spectrum was reached. Then the order was reversed, presenting those wavelengths that had been skipped.

For each wavelength, stimulus irradiance was varied in 1.0 log unit steps using an ascending method of limits procedure until an initial ERG b-wave response was obtained. Once this irradiance value was determined, stimulus irradiances were presented around this value using an ascending method of limits procedure. Irradiance was incremented in 0.5 log unit steps until the b-wave amplitude was larger than +50 microvolts. For each trial, stimulus wavelength, log attenuation, and background adaptation intensity were entered into the laboratory computer. To begin any trial, the stimulus shutter was opened via the laboratory computer and the test stimulus was superimposed onto the white adaptation light

for 200 ms. ERG responses were then sent to the computer, displayed graphically on the computer's monitor, and stored on disk for later analysis. Baseline recording was done for 50 ms prior to stimulus presentation. Also, recordings were made for 750 ms following stimulus presentation. Thus, ERG responses were recorded for total of 1000 ms. Each trial consisted of three consecutive stimulus presentations for a total of three ERGs for a given wavelength at a given intensity. After presenting stimuli at this adaptation level, fish were adapted to the other background light for 20 minutes and then tested under this background. The order of the background adaptation level was varied across sessions.

The above description outlines animal preparation and experimental procedures for one fish for one test session. Each fish served under both levels of light adaptation. When a particular test session was completed, the experimenter attempted to revive the fish. If it was not possible to revive a fish, then the fish was sacrificed using standard procedures.

Chapter 3

Results

The first issue in this study concerned whether color and luminance would be processed separately in the zebrafish visual system. To determine whether separate functional visual channels exist for color and luminance, spectral sensitivity functions based on ERG b-wave responses were derived under two levels of background adaptation. It was hypothesized that if zebrafish do process color and luminance separately, the two different levels of background adaptation used in this project should yield qualitatively and quantitatively different spectral sensitivity functions. Under the low white background adaptation (0.0053 mW/cm^2 or -6.0 log units attenuation), spectral sensitivity functions were predicted to be smooth and broad-band, which is indicative of the luminance channel. Under the high level of white background adaptation (4.36×10^2 mW/cm^2 or -1.0 log units attenuation), it was predicted that the spectral sensitivity function would show several notches and dips which are characteristic of the chromatic channel.

Preliminary Data Analysis

Electroretinogram responses. The dependent measure in this study was the amplitude of the b-wave component of the electroretinogram (ERG). An example of a zebrafish ERG is given in Figure 1a. The three ERGs in this figure were obtained with a 400 nm stimulus at -2.0 log unit attenuation under the high background adaptation. The raised bars in Figure 1 mark the onset and termination of the stimulus. Since in recording ERGs it is necessary to amplify the electrical signal 10,000 times, electrical noise from laboratory equipment also can be recorded. Therefore, the ERG signal may contain

unwanted electrical noise in addition to the actual physiological responses from the retina. Prior to analysis, the ERG data were filtered for 60 Hz noise; 60 Hz input is typically the result of electrical noise.

To remove 60 Hz noise from the ERG response, a moving averaging software filter was used (Gates & Becker, 1989). This filter averages a small number of points together in a series to produce a new data point. Thus, a particular data point is averaged with several nearby data points, without a reduction in the number of data points (Gates & Becker, 1989). The moving averaging filter used in this project was a notch filter (Vennat, Besse, Sanzelle, Doly, & Gaillard, 1994). A notch filter minimizes the response at a specific frequency. In this study, the notch filter was set at 60 Hz; this would remove electrical noise in the ERG response, while not affecting the actual ERG signal. Filtering was done off-line at the conclusion of a test session. Figure 1b illustrates the same three ERGs shown in Figure 1a after they were filtered for 60 Hz noise. Examining this figure, it is clear that filtering does not distort the shape or magnitude of the ERG components, but does reduce unwanted electrical noise.

After being filtered, ERGs were averaged across the three trials. To average the three ERGs, it first was necessary to set each baseline response prior to the ERG signal to zero. It is important to do this when averaging since when recording consecutive ERGs, there is a tendency for the responses to drift. Although each single ERG might be similar in its amplitude, the baseline amplitudes, due to drift in the electrical signal, might begin at different levels. If ERGs are not first set to zero, then the averaging would incorporate differences in baseline amplitude. To set the ERG response to zero, the average baseline amplitude for a single ERG was subtracted from every data point for that ERG. This zeroing procedure was done for all three ERG responses and then the three ERGs for a single trial were averaged.

Although the ERG response to a single test stimulus could have been used as a measure of the retinal response, it was difficult to determine b-wave amplitude for ERGs obtained with stimuli of very low irradiance (see below). At low stimulus irradiances, averaging the response to the three stimulus presentations provided a more representative sample of the b-wave amplitude at a particular stimulus irradiance. For example, averaging the ERGs shown in Figure 1b produces the ERG shown in Figure 2a. Note that averaging reduces noise even further but does not affect the ERG b-wave response.

Figure 2b shows averaged ERG responses to a 400 nm stimulus at different stimulus irradiances. It is clear that the amplitude of the b-wave changes as a function of stimulus irradiance. At a high stimulus irradiance such as -2.0 log units, the b-wave response (labeled -2.0) is very large exceeding 200 microvolts (this is the same ERG shown in Figure 2a). As stimulus irradiance is decreased to -2.5 log units, the amplitude of the b-wave decreases as well. Note that without response averaging, the smaller b-wave signals would be hidden in the electrical noise. It should be clear from this figure that the b-wave amplitude varies with stimulus irradiance, and therefore, the b-wave is a useful measure to assess a visual system's sensitivity to different wavelengths of light at various irradiances. Note that at -4.0 log unit attenuation, there does not appear to be any b-wave component.

The researcher initially proposed to measure the response of the ERG a-wave as well. After considerable examination, it was decided not to continue using this response for two main reasons. First, the actual a-wave response was not consistently large enough to detect even after response averaging. The a-wave response is a small negative response at stimulus onset and the intensity of the two background adaptations used might not be adequate to elicit an a-wave response. Second, there might have been too much electrical

noise present to detect the small a-wave response. In this case, any true physiological response would be meshed with electrical noise and it was impossible to reliably distinguish them.

Irradiance-response functions. The increase or decrease of the b-wave amplitude relative to stimulus irradiance is best illustrated in the irradiance-response function. Figure 3a shows an irradiance-response function for the averaged ERGs from Figure 2b. Irradiance-response functions plot the maximum b-wave response amplitude (in microvolts) as a function of the log irradiance of the monochromatic light in quanta/cm^2/s. To determine the maximum b-wave response for each stimulus irradiance, the averaged ERG responses to a stimulus of a given wavelength were put into a computer spreadsheet. Once the ERG data were in a spreadsheet, maximum b-wave amplitude at each intensity was easily determined by using a command (i.e., sort or maximum/minimum) to find the maximum value of the averaged ERG. For example, the averaged ERG from Figure 2a produced a maximum b-wave response amplitude of +225 microvolts. Figure 3b shows an irradiance-response function for a 600 nm stimulus, also at the higher background adaptation

For each irradiance-response function, the stimulus irradiance which produced a criterion response was determined. For this study, the criterion response for the ERG b-wave response was +50 microvolts (Demarco & Powers, 1989). To determine the criterion response from an irradiance-response function, linear regression was used to interpolate to find the stimulus log irradiance that would produce a +50 microvolt response. Each irradiance-response function here illustrates very well that as the irradiance of the monochromatic stimulus increases, the amplitude of the b-wave increases. Note that even though Figures 3a and 3b are similar in their shape, the log relative irradiance needed to elicit a +50 microvolt criterion response at 400 nm (-2.72) is different from the irradiance

needed to elicit a +50 microvolt response for 600 nm (-2.16). It is important to note that these irradiance values are relative to the irradiance emitted at 0.0 log attenuation (no attenuation) for each wavelength (see Appendix A). For example, referring to the functions depicted in Figure 3, a different number of quanta are needed to elicit the same criterion response of +50 microvolts for the two wavelengths. That is, for 400 nm (Figure 3a), a log relative irradiance of -2.72 is equal to 9.74 quanta/cm^2/s whereas for 600 nm, -2.16 log relative irradiance is equal to 10.79 quanta/cm^2/s. This means that more light is needed (about 1.0 log units more) for a 600 nm stimulus than for a 400 nm stimulus in order to elicit the same criterion response of +50 microvolts. Therefore, the visual system is more sensitive to a 400 nm stimulus than a 600 nm stimulus.

Spectral Sensitivity Function

The major investigative tool in this project to examine parallel mechanisms in the zebrafish was the spectral sensitivity function. To obtain a spectral sensitivity function, the stimulus irradiance which produced a b-wave criterion response of +50 microvolts was determined for each monochromatic stimulus used for a given white background condition. The sensitivity to each stimulus wavelength was found by calculating the reciprocal of the log stimulus irradiance which produced the criterion response. Spectral sensitivity functions were produced by plotting the reciprocal of the log stimulus irradiance at each wavelength. For all spectral sensitivity functions, this was accomplished by simply inverting the y-axis scale. Thus, the most sensitive response requires less light; the smaller the value on the y-axis, the more sensitive the response. Figure 4 shows a spectral sensitivity function derived from one subject under the high level of white background adaptation (-1.0 log unit attenuation). Figure 4 illustrates several important features about the spectral sensitivity function under this background condition. First, it is clear that this

animal is not equally sensitive to all wavelengths, and second, this animal is more sensitive to short than to long wavelengths of light.

Figure 5 illustrates the average spectral sensitivity functions for ten fish, derived under the low (triangles) and high (squares) white background adaptation. Data from three fish were not included in the average spectral sensitivity functions or any statistical analyses since complete spectral sensitivity data were obtained under only one background condition. Examining the two spectral sensitivity functions in Figure 5 qualitatively, there does appear to be a difference in their shape. The top curve shown in Figure 5 is the spectral sensitivity function derived under the low background adaptation (-6.0 log unit white background). This curve shows a dramatic decline in sensitivity around 400 nm which is suggestive of opponent mechanisms. In addition, spectral sensitivity at the low background level clearly shows several peaks in sensitivity; the wavelengths to which these peaks occur correspond to the l-max's of the four cone types in zebrafish (360, 420, 480, and 560 nm) (Robinson et al., 1993).

Comparatively, the bottom curve shown in Figure 5 shows the spectral sensitivity function derived under the high background adaptation (-1.0 log unit white background). This spectral sensitivity function appears to be smooth and broad-band, with no large declines in sensitivity. There appear to be peaks at 360, 420, and 480 nm, which correspond to the l-max's of the U-, S-, and M-cone photopigments; however these peaks are not as apparent as in the spectral sensitivity function under the low background adaptation. Furthermore, there does not seem to be a prominent peak at 560 nm under this condition which is the λ-max of the L-cones (Robinson et al., 1993). It also is evident that sensitivity is greater under the low level of background adaptation.

In addition to qualitative differences between the two spectral sensitivity functions shown in Figure 5, there are quantitative differences as well. A 2 (background) x 13

(wavelength) repeated measures analysis of variance (ANOVA) showed a main effect of background adaptation, $F(1,9)=105.74$, $p<.005$, as well as a main effect of wavelength, $F(12,108)=126.05$, $p<.005$. Furthermore, a significant interaction between background condition and wavelength was found, $F(12,108)= 16.31$, $p<.005$. A simple effects analysis (Keppel, 1991) of background condition at each wavelength showed that sensitivity under the low background level was significantly greater than sensitivity under the high background level at every wavelength ($p<.005$).

Manipulation Checks For Order Effects

Since it is possible that the order in which stimuli were presented could confound the results, presentation order was varied across test session (see Procedure). To ensure the integrity of the spectral sensitivity functions obtained, spectral sensitivity functions were derived comparing sessions in which stimulus presentations began at 320 nm (triangles) and 660 nm (squares) (Figures 6a and 6b). Each spectral sensitivity function in Figures 6a and 6b is based on five test sessions. Note that for each background condition, the two spectral sensitivity functions have similar shape and, in fact, almost fall on top of one another. Thus, under both background conditions, spectral sensitivity is very similar regardless of which end of the spectrum stimulus presentations began. Therefore, there were no order effects within a given background condition.

The order fish were adapted to the background light could affect the spectral sensitivity function as well. Figure 7a shows two spectral sensitivity functions; one is based on seven sessions under the -6.0 log unit white background in which fish were first adapted to -6.0 log unit white background (triangles). The other spectral sensitivity function is based on three sessions under the -6.0 log unit white background after fish were first adapted to the -1.0 log unit white background (squares). There does not appear to be

any order effects evident in Figure 7a. The shapes of the two functions are similar and the two functions virtually superimpose onto one another. Figure 7b shows two spectral sensitivity functions derived under the -1.0 log unit white background. One function is based on seven sessions in which fish were first adapted to the -6.0 log unit white background (triangles). This function is compared to a spectral sensitivity function based on three sessions where fish were first adapted to the -1.0 log unit white background (squares).

There appear to be some order effects present under the -1.0 log unit white background as shown in Figure 7b. The spectral sensitivity function obtained when the subject was exposed to the -6.0 log unit white background first was slightly more sensitive than when the subject was exposed to the -1.0 log unit white background first. However, these effects are not that surprising and do not distort the data presented thus far. For instance, notice that both spectral sensitivity functions in Figure 7b are similar in shape. Recall that it is the shape of the spectral sensitivity function that is important in determining whether separate channels for color and luminance exist. One reason for changing the order of the adaptation background was related to fish integrity as a test session lengthened. However, Figure 7b shows that the validity of the data did not suffer due to fish health. The top curve represents the spectral sensitivity function for seven fish first adapted to -6.0 log unit white background before being adapted to the -1.0 log unit white background. Notice that sensitivity under the -1.0 log unit white background is better (i.e., more sensitive) after first being adapted to the -6.0 log unit white background. Thus, it is not likely that declining fish health accounted for the differences in sensitivity presented in Figure 7b.

Cone Contribution to Spectral Sensitivity Functions

The second hypothesis predicted that spectral sensitivity functions derived under the high and low levels of background adaptation would be composed of different combinations of cone types. Specifically, it was predicted that the spectral sensitivity function under the low level of background adaptation would have additive cone input, with contributions from at least the middle and long wavelength cones. Under this condition, the contribution to the spectral sensitivity function from any cone should be positive. It also was predicted that ultraviolet cones would contribute to the spectral sensitivity function under the low background level. Finally, it was predicted that under the high level of background adaptation, sensitivity would dramatically decline at some wavelengths; therefore, the spectral sensitivity function would be composed of both additive and subtractive cone input.

To determine cone contributions to the spectral sensitivity function under the different adaptation levels, a linear model was applied to the spectral sensitivity data (DeMarco & Powers, 1991; Coughlin & Hawryshyn, 1994a). This model assigns positive weights for excitatory responses and negative weights for inhibitory responses. This model made it possible to determine the relative input weight from each cone type to the zebrafish spectral sensitivity function under the low and high levels of light adaptation. The model used for this study is given below:

$$S_l = (K_U * A_U) + (K_S * A_S) + (K_M * A_M) + (K_L * A_L)$$

where S_l = Relative spectral sensitivity of a fish at wavelength l

A_X = The relative absorptance for cone type x at wavelength l

K_X = The weight coefficient for cone type x

Absorptance spectra were derived from nomograms based on the rhodopsin molecule. Dartnall (1953; cited in Knowles & Dartnall, 1977) noted that the shapes of the

absorption spectra for different photopigments were similar if absorptance was plotted as a function of frequency rather than wavelength. Given this observation, nomograms for the cone types found in the zebrafish retina were constructed based on their λ-max derived using microspectrophotometry (Robinson, et al., 1993). Analyses were performed to determine whether nomograms should be based on the rhodopsion or porphyropsin molecule. This test was done since the porphyropsin molecule is generally used to construct nomograms in freshwater fish (Davson, 1977). A χ^2 goodness of fit test showed that the nomograms based on rhodopsin (χ^2=2.54, df=13, $\underline{p}$ <.99) provided a better fit to the actual data from the zebrafish ultraviolet cone pigment (Robinson et al., 1993) than did the nomograms based on porphyropsin (χ^2=13.09, df=13, $\underline{p}$ <.50).

To determine the relative percent absorptance at each wavelength, an eighth order polynomial was applied to the zebrafish cone absorptance spectra to produce a best fitting curve for that data (Flamarique & Hawryshyn, 1996). The resulting best fitting curve was then used to derive the log relative absorptance of each cone type for the wavelengths used in this study; the absorptance data were converted to percent relative absorptance and normalized on a 0 to 1 scale (see Figure 8). Spectral sensitivity data derived from ERG b-wave responses were converted to percents and normalized on a 0 to 1 scale as well.

Modeling procedures were done using StatMost, version 2.5 (Data Most Corporation, Salt Lake City, UT). To use the curve fitting program, the normalized spectral sensitivity data, the normalized relative absorptance data, and the initial weights to the linear model were entered into a nonlinear regression program which used a least-squares-curve-fitting-Simplex-algorithm. In nonlinear regression, initial weights for K_X must be given to begin the program. Based on these initial weights, the program performed a specified number of iterations to minimize residual error using a least squares procedure. However, it is important to note that since the iteration begins with a user

defined set of values, the final values for the parameters may be different for a different set of initial values. Ideally, it is desirable to compare relative weights after the last iteration for several different sets of data with different starting weights; thus it is possible to see whether on each occasion the final weights are similar. Theoretically, valid weights should not vary considerably with different starting weights. Different values for the starting weights were entered into the curve fitting program to determine whether after the iteration procedures, the final weights would differ depending upon the starting value. It was found that changing the initial parameters did not produce results different from those when the starting weights were 0.0. Therefore, for this study, the starting weight for each cone type was 0.0. Modeling procedures were done for the spectral sensitivity data from ten fish, as well as the average spectral sensitivity data, at the low and high background adaptation levels.

After performing the modeling procedures on a set of normalized sensitivity data, the computer program generated the sensitivity data predicted by the model based on the final parameters of the equation. Table 1 shows the relative cone weights computed for ten fish at both levels of background adaptation. Table 1 also shows the weights computed on the average relative spectral sensitivity data under both background levels. Additionally, the sums of squares of the cone weights for each subject, as well as the averaged spectral sensitivity data, are provided in Table 1. The average relative spectral sensitivity data (squares) for the high background level plotted with the spectral sensitivity data predicted by the model (solid line) are shown in Figure 9. In addition, the model weights for each cone type are given. The sensitivity data predicted by the model fit well with the actual relative sensitivity data ($r = .94$, $df=9$, $p<.01$). Looking at the cone weights, the U-cones made the greatest contribution to the average spectral sensitivity function under the high background level (+0.95). Figure 9 also shows that the M-cone has the second largest input weight (+0.14), followed by the S-cones (+0.09) and finally the L-cones with the

smallest weight coefficient (+0.02). Furthermore, all weights for the spectral sensitivity function under the high background level are positive.

Comparatively, Figure 10 shows the average relative spectral sensitivity data for the low background level (squares) plotted with the spectral sensitivity data predicted by the model (solid line). Again, the final weights for each cone type are given. A goodness of fit test showed that the sensitivity data predicted by the model fit well with the actual sensitivity data (r= .88, df=9, p<.01). Under the low background adaptation, the M-cones made the largest contribution to the average spectral sensitivity function (+0.74). The U-cones made the second largest contribution (+0.64), and then the S-cones with a negative contribution (-0.23). Finally, the L-cone contribution was +0.13.

Figure 11 shows the mean relative cone weights from the spectral sensitivity functions of ten fish under both background adaptations (see Table 1). Looking at Figure 11, the contribution of a particular cone type easily can be compared at each background adaptation. Under both background conditions, the U-cones make a large contribution, but the weight is greater under the high background adaptation. However, the relative weights of M-and L-cones are larger under the low background adaptation. Furthermore, the S-cone weight is negative under the low background adaptation relative to its positive input under the high background adaptation. Note that although the weight of the S-cones is negative, they contribute more under the low background adaptation than under the high background adaptation, although the contribution is inhibitory.

To determine if the contributions of the four cone types differed significantly at the high and low background adaptation, an ANOVA was performed on the relative cone weights with background condition (high and low) and cone type (U, S, M, and L) both as repeated factors. The ANOVA showed a main effect of cone type, $F(3,27)= 73.13$, $p<.005$, and a significant background x cone type interaction, $F(3,27)=26.02$, $p<.005$.

There was no difference in relative cone weights across the high and low background conditions ($p=.414$). Four simple effects analyses (Keppel, 1991) comparing each cone type across the two background adaptations found that the relative weight of each cone type differed significantly across the low and high background adaptations ($p<.01$).

Chapter 4

Discussion

The first issue addressed in this project was whether the processing of color and luminance information would be represented by separate channels in the zebrafish visual system. It was predicted that under the low level of white background adaptation, the spectral sensitivity function would be smooth and broad-band, which is representative of the luminance channel in higher vertebrates. It also was predicted that under the high level of white background adaptation, the spectral sensitivity function would show several notches and dips which are characteristic of the chromatic channel. The second issue concerned which of the four cone types in zebrafish would contribute to spectral sensitivity functions derived under the high and low white background adaptations. It was hypothesized that the spectral sensitivity function under the low level of white background adaptation would have additive cone input, with contributions from at least the M- and L-cones. In addition, it was predicted that the ultraviolet cones would make a contribution under the low level of white background adaptation. Under the high level of white background adaptation, it was predicted that there would be both additive and subtractive cone input to the spectral sensitivity function.

Summary of Results

The spectral sensitivity function of the zebrafish was found to be different under the high and low levels of white background adaptation. The spectral sensitivity function under the low level of white background adaptation showed a large decline in sensitivity around 440 nm; such a drop in sensitivity is indicative of color opponency in primates (Zrenner et al., 1990). Therefore, the spectral sensitivity function obtained under the low

white background adaptation appears to represent a chromatic channel (see below). In addition, this spectral sensitivity function possessed four peaks in sensitivity; these peaks corresponded to the l-maxs of the four cone photopigments found in the zebrafish retina.

Conversely, under the high level of white background adaptation, the spectral sensitivity function was smooth and broad-band, showing no evidence of opponent mechanisms. The spectral sensitivity function under the high level of white background adaptation also showed peaks in sensitivity which corresponded to the l-maxs of the cone photopigments in zebrafish. However, there did not appear to be a prominent peak in sensitivity in the long wavelength region of the spectrum. In addition, under the high level of white background adaptation, the spectral sensitivity function was less sensitive than under the low level of white background adaptation. At every wavelength of light used, sensitivity was greater under the low level than under the high level of white background. This finding would be expected since adapting the visual system to a very intense white background makes the visual system less sensitive (Boynton, 1979).

The relative contributions of the four cone types differed for the spectral sensitivity functions derived under the high and low levels of white background adaptation. The spectral sensitivity function obtained under the low level of white background adaptation did have contributions from the M- and L-cones, but there also was an S-cone contribution which was negative. In fact, based on the model weights, the contribution of the S-cones was greater than that of the L-cones. Comparing the relative contribution of the M- and L-cones, the M-cone contribution was about six times that of the L-cones. Under the low background adaptation, the ultraviolet cones made a large contribution; compared to the other cone types, the U-cones made the biggest contribution to the spectral sensitivity function. Under high background adaptation, all cone contributions were positive, with the greatest contribution from the U-cones. The M-cones made some contribution but not as much as the contribution made by the U-cones. The large

contribution made by the U-cones under both background conditions is not surprising given the large population of U-cones in the zebrafish retina. Robinson et al. (1993) report that U-cones constitute roughly 25% of the cone population.

Interpretation of Major Findings

Low white background adaptation. The most interesting finding of this study was that the low level of white background adaptation produced a spectral sensitivity function similar to the spectral sensitivity function of the chromatic channel found in higher vertebrates. It was originally predicted that this level of white background adaptation would yield a spectral sensitivity function indicative of a luminance channel; the spectral sensitivity function under this background condition was predicted to be smooth and broad-band. This hypothesis was not supported by the data. In fact, the result was exactly opposite from that originally predicted. As described above, the spectral sensitivity function obtained under the low level of white background adaptation showed a large drop in sensitivity around 440 nm, suggesting the presence of opponent mechanisms.

Finding evidence to suggest zebrafish may possess a chromatic channel under this background condition is not congruent with data reported in the current literature on chromatic channels in higher vertebrates. First, it is important to note that the intensity of the low level of white background used in this study would not yield a spectral sensitivity function indicative of a chromatic channel with higher vertebrates (King-Smith & Carden, 1976; Mills & Sperling, 1990). Rather, such a low intensity level would produce a broad-band spectral sensitivity function. It is not clear why the low level of white background adaptation produced a spectral sensitivity function which is representative of opponent mechanisms. However, the results of this study indicate that the zebrafish may possess a chromatic channel, one that is qualitatively similar to the chromatic channel of higher vertebrates.

There are several pieces of evidence suggesting that the spectral sensitivity function obtained under the low level of background adaptation represents a chromatic channel. For example, this function qualitatively resembles the spectral sensitivity function of the chromatic channel in higher vertebrates. A number of studies cite evidence for chromatic mechanisms based on the notches present in the spectral sensitivity function. The notch which is found between two peak sensitivities is the result of opponent processing in the visual system. For example, Mills & Sperling (1990) discuss opponent mechanisms in the macaque spectral sensitivity function which was derived from ERG b-wave responses. There were notches, even though the magnitude of these notches was relatively small. Other studies that discuss opponent mechanisms in the spectral sensitivity function report larger dips in sensitivity (i.e., larger notches, Sperling & Harwerth, 1971). There really is no agreement to how large a decline in sensitivity is required to be considered the product of opponent mechanisms. For this study, the spectral sensitivity obtained under the low level of white background adaptation possessed a large notch around 440 nm. Thus, at least visually, the spectral sensitivity function obtained under the low level of white background adaptation is similar to the spectral sensitivity function of the chromatic channel in higher vertebrates.

Another important finding suggesting that a chromatic channel was found under the low level of white background adaptation comes from the results of the computational modeling. A negative weight for the S-cones was found under the low level of white background adaptation, which implies there is some inhibition between different regions of the spectrum. Linear models, such as the one used in this study, permit the different cone input signals to a spectral sensitivity function to be summed algebraically, and therefore they have been useful in determining opponent interactions between different cone types in higher vertebrates (Sperling & Harwerth, 1971) and some fish species (DeMarco & Powers, 1991; Coughlin & Hawryshyn, 1994a). For instance, under the low level of

white background adaptation, there were large U- and M-cone contributions; however, there also was a large negative weight for the S-cones. Therefore, not only was there a large decline in sensitivity around 440 nm, this dip also was accompanied by a negative cone weight for the S-cones.

Finally, further evidence of a chromatic channel is found if the known absorptance spectra for the four cone types in zebrafish (Figure 8) are compared with the relative spectral sensitivity function under the low level of background adaptation (Figure 10). If Figure 8 is superimposed onto Figure 10, it is easy to see how the obtained spectral sensitivity data fit the absorptance spectra for a particular cone type. For example, if one compares the sensitivity data from the long wavelength region (Figure 10) with absorptance spectra for the L-cones (Figure 8), there is a relatively nice fit. In other words, the sensitivity data obtained at the longer wavelengths are appropriate given what would be expected based on the absorptance spectra of the L-cones. If the same is done for the M-cone absorptance spectra, a different picture emerges. For example, notice that the obtained spectral sensitivity data for the middle wavelength region is considerably narrower that the M-cone absorptance spectra.

A similar result was found in a study by Neumeyer (1984) in which the absorptance spectra of goldfish did not fit the behavioral spectral sensitivity function very well. She found that the peaks of the spectral sensitivity function were much narrower than the absorptance spectra for the three cone types found in goldfish. In addition, the behaviorally determined spectral sensitivity function was shifted toward the longer wavelengths compared with the absorptance spectra. Other evidence from the Neumeyer study suggested that the spectral sensitivity function was driven by spectrally opponent cone mechanisms. Neumeyer used chromatic adaptation to help determine if some neural interaction (i.e., opponency) was the reason for the discrepancy found between the spectral

sensitivity data and the absorptance spectra. By chromatically adapting with long, middle, and short wavelengths, it was possible to selectively suppress each of the three cone types and then examine the spectral sensitivity function. She found that the shape and location of the peak sensitivities changed under the three different conditions of chromatic adaptation. Neumeyer suggested that if the three cone types were in a sense "additive," this would not occur; there would only be a suppression of sensitivity in the wavelength region corresponding to the cone type being adapted. Thus, according to Neumeyer, some neural interaction in the form of opponent mechanisms among the three cone types in goldfish account for narrowing of the peaks of the spectral sensitivity function.

Additionally, it is unlikely that a lower level of white background adaptation would be better for detection of a luminance channel. It is important to note that the luminance channel in zebrafish may have a lower threshold for activation than that reported in higher vertebrates. However, the intensity of the lowest background condition was as low as it could be and still be in the range for photopic vision (cone vision) in zebrafish (Branchek, 1984). Using a lower intensity background to isolate the luminance channel could result in rod contribution to the response. In fact, it is possible that the intensity of the light used at the low level of background was so low as to include rod contribution, since the absorptance spectra of the rod and M-cone photopigments are similar. However, it is doubtful that rods were contributing to the function since rod vision typically is much more sensitive than what was found in this study (Mackintosh et al., 1987). Therefore, it is unlikely that the intensity of the white background adaptations used in this study explain the failure to find a luminance channel in zebrafish.

When examining literature which has used lower vertebrates such as fish, the results of this study both support and contradict the existing literature. For example, Regan, Schellart, Spekreijse, and Van Den Berg (1975) used a procedure known as heterochromatic flicker photometry (HFP) to derive spectral sensitivity functions based on

ERG responses in goldfish. The HFP procedure takes advantage of another property which distinguishes chromatic and luminance channels in higher vertebrates, which is temporal rate. They found that using HFP under three different background intensities (over a 2.0 log unit range) did not change the shape of the spectral sensitivity functions. Spectral sensitivity functions under each background were smooth and broad-band; they differed only in their absolute sensitivity. Thus, Regan et al. found no evidence for a chromatic channel in the goldfish.

However, Patterson (1996) used HFP to try to isolate chromatic and luminance channels from ERG responses in zebrafish. To isolate chromatic and luminance channels, he flickered monochromatic light and a white reference light at two different frequencies (4.6 and 16 Hz). Unlike Regan et al. (1975), the spectral sensitivity functions Patterson obtained were not smooth and broad-band; for both 4.6 and 16 Hz flicker, spectral sensitivity functions showed subtle signs of opponent mechanisms. In fact, the spectral sensitivity function derived with 4.6 Hz flicker closely resembled the spectral sensitivity function from this study obtained under the low level of white background condition. Although the results were very subtle compared to those of the present study, Patterson noted that there was a lot of variability in the data, due in part to the data analysis used in the HFP procedure. He suggests that reducing this variability would provide stronger evidence for a chromatic channel using HFP in zebrafish. Furthermore, the HFP procedure may not be as sensitive as the increment threshold technique.

Recent evidence from single unit studies of fish suggest that the visual systems of certain fish species possess neurons which exhibit color opponency (Coughlin & Hawryshyn, 1994a). In fact, Mackintosh et al. (1987) and Bilotta & Abramov (1989a, 1989b) show that goldfish ganglion cells can be classified as spectrally opponent. Like single unit studies, the results from this study provide evidence for a chromatic channel;

such a chromatic channel would be driven by spectrally opponent ganglion cells like those found in single unit recording studies.

High white background adaptation. The second major finding from this study was that the spectral sensitivity function obtained under the high level of white background adaptation was smooth and broad-band. Initially, it was hypothesized that the high level of white background would produce a spectral sensitivity function that showed notches, or declines in sensitivity at some wavelengths due to opponent mechanisms. This hypothesis was not supported by the data. The obtained spectral sensitivity function was exactly opposite than that originally hypothesized.

Comparing the spectral sensitivity function obtained under the high level of white background adaptation with research from higher vertebrates, some discrepancy is found. The intensity of the white light used at the high background condition in this study would not yield a spectral sensitivity function which resembles a luminance channel in higher vertebrates (King-Smith & Carden, 1976). With the primate visual system, such bright conditions are considered ideal for chromatic processing; the spectral sensitivity function, whether it is behaviorally or electrophysiologically derived, clearly shows opponent mechanisms (King-Smith & Carden, 1976; Mills & Sperling, 1990; Sperling & Harwerth, 1971). The spectral sensitivity function found under the high level of white background adaptation in no way qualitatively resembles the spectral sensitivity function of the chromatic channel in the primate.

The spectral sensitivity function obtained in this study under the high level of white background adaptation appears to represent a luminance channel; it is unlikely, however, that it represents a luminance channel for several reasons. The role of the luminance channel is to detect light under low levels of illumination; in higher vertebrates, the cone signals of M- and L-cones are additive to enhance luminance detection (Zrenner et al., 1990). However, the intensity of the high level of white background adaptation in this

study was equal to normal daylight conditions. Therefore, these conditions were extremely bright and ideal for color vision. Conceptually, it is difficult to understand why a system whose job is to detect luminance under low light levels would be needed at such a high level of illumination.

A better explanation is that zebrafish possess only one channel, a chromatic one, and that the intensity of the higher white background simply suppressed the sensitivity of the cone contributions, including the "inhibitory" S-cone component, compared to the lower background condition. Examining the cone weights under the two levels of white background adaptation, there is evidence for the suppression of sensitivity of the cone types under the high background condition (see Table 1). For example, in going from the low to the high white background adaptation, there is a decrease in the weights of the all cone types except for the U-cones. Note that the S-cone contribution to the model changes from a strong inhibitory component (-0.23) under the low level of white background, to just about zero (+0.09) under the high level of white background. In addition, the M- and L-cone contributions are reduced under the high level of white background, compared to their contribution under the low level of white background. The fact that the weight of the U-cones is greater under the high level of white background adaptation is not surprising. Due to the spectral distribution of the white background light, it will affect the U-cones less than the other cone types. By selectively reducing the input of the other cone types, the U-cones appear to contribute more to the response.

Based on the spectral sensitivity function obtained under the high level of white background (as well as the one obtained under the low level of white background), it seems that the zebrafish does not possess a luminance channel. This finding is not surprising based on the primate literature. As with the chromatic channel, a separate pathway exists in primates for transmitting luminance information. Although zebrafish may possess a

chromatic channel, it could be argued that zebrafish cannot have an additional channel for luminance since they do not have separate anatomical pathways for color and luminance like higher vertebrates. Therefore, it is necessary to have anatomically separate neural pathways which subserve color and luminance information in order to process color and luminance separately.

A number of studies report that fish possess the capability to process luminance information separately from chromatic information. For example, spectrally nonopponent cells have been found in goldfish (Mackintosh et al., 1987; Bilotta & Abramov, 1989a) and in trout (Coughlin & Hawryshyn, 1994a). These cell types show the same type of response (i.e., excitation or inhibition) to all wavelengths of light; therefore, there is no indication of opponent mechanisms in spectrally nonopponent cells. It is interesting that no evidence of a luminance channel was found in this study, yet luminance detecting cells have been reported in other fish. It is important to keep in mind, that at present, there is no single unit data available for the zebrafish. Thus, it is possible that the zebrafish does possess both spectrally opponent and nonopponent cells.

The discussion thus far has focused on the finding that the zebrafish appears to possess only a chromatic channel and no luminance channel. It might be argued that the particular methodology used in this study could account for the failure to find separate chromatic and luminance channels in zebrafish. This researcher used an increment threshold procedure with the ERG, whereas studies such as those of Coughlin and Hawryshyn (1994a) used an increment threshold technique with single unit recordings. However, an increment threshold technique has been used to show both chromatic and luminance channels with higher vertebrates using the ERG (Mills & Sperling, 1990). Interestingly, only in higher vertebrates do single unit and ERG data agree to suggest separate channels for color and luminance. It is important to note that the ERG and single unit recordings represent data from different levels of the retina. Single unit studies are

performed on retinal ganglion cells, whereas the ERG probably samples electrical responses from the bipolar cells (there is still some uncertainty as to which cells make the most contribution to the vertebrate ERG). Possibly, the zebrafish does not possess spectrally nonopponent bipolar cells like some other fish species (Nicole, 1989). This observation would account for not finding a luminance channel in the zebrafish at the level of the bipolar cells.

Ultraviolet cone contribution. The final major finding from this study was the importance of the ultraviolet cones (U-cones) in visual processing in zebrafish. Under both the high and low levels of white background adaptations, U-cones made a substantial contribution to the spectral sensitivity functions. This study has been one of only two (see Patterson, 1996) which address the role of the U-cones in zebrafish vision. This study demonstrates that zebrafish rely heavily upon the contribution of the U-cones for vision.

The large contribution of the U-cones to the ERG response is not too surprising given that this cone type constitutes roughly 25% of the cone population in zebrafish (Robinson et al., 1993). Due to this large number of U-cones, it would be expected that the U-cones play an important functional role for vision in zebrafish. There are several possible reasons why the U-cones are so important to zebrafish vision. For example, one reason might be related to the fact zebrafish are considered "top dwellers." There is much more sunlight, even ultraviolet light, found in shallower waters than at deeper depths. Zebrafish remain "top dwellers" throughout their lifespan, and therefore constantly come in contact with ultraviolet light. Thus, it is to their advantage to keep their U-cones even as adults. What is interesting is that most fish which possess U-cones lose these cones at adulthood. For example, trout possess U-cones as juveniles but lose the U-cone type in early adulthood (Goldsmith, 1994). The fact that their environment changes as they reach adulthood may account for the loss of the U-cones. Adult trout (and other salmonids; see

Hawryshyn & Harosi,1994) spend most of their adult life in deeper waters where there is little ultraviolet light available; however, when young, they are found in shallow streams. Therefore, juvenile trout rely on the U-cones for vision but adult trout do not.

Since the results of this study suggest that only a chromatic channel is present in zebrafish, it is difficult to speculate on the role of the U-cones for luminance processing, or compare the results of this study with other studies. But a series of studies by Coughlin & Hawryshyn (1994a,1994b) suggest that U-cones do contribute to both chromatic and luminance processing. Coughlin & Hawryshyn (1994b) used the same modeling procedures this study used and found that U-cones contributed to the spectral sensitivity functions of individual neurons in the cortex of juvenile rainbow trout (*Oncorhynchus mykiss*). For cells which exhibited spectral opponency, there was additive and subtractive cone input whereas cone input to luminance cells was positive. The present study also found that U-cones contribute to the spectral sensitivity function of the chromatic channel.

General Implications of Findings

The results presented here permit a comparison to be made between visual processing in the zebrafish and what is known from extensive research related to parallel visual processing in primates. Research investigating separate visual channels has shown that the most efficient way for a visual system to encode the many qualities of a visual scene is to transmit these qualities along separate, parallel visual channels. Parallel processing theory would predict that a vertebrate such as the zebrafish would not process chromatic and luminance information separately given they lack anatomically separate pathways for color and luminance. At least in zebrafish, a single channel is present which is designed to process chromatic information. The results of this study provide further evidence advocating the need for separate visual pathways for separate color and luminance processing.

It is important to note that the results of this study only suggest that zebrafish do not process color and luminance information separately. Other studies do show that lower vertebrates such as fish possess anatomically separate pathways responsible for other information. For example, in goldfish, it is well established there are anatomically separate ON and OFF pathways (DeMarco & Powers, 1991; Nicole, 1989) for processing increments and decrements of light. Compared with primates, visual systems such as that of goldfish are much less specialized, although they function adequately and are comparable to primate visual systems in a number of visual functions. Bilotta & Abramov (1989b) showed that separate visual pathways for certain spatial properties and color information are not required in goldfish as in primate visual systems. For example, they showed that both spectrally opponent and nonopponent ganglion cells were sensitive to high spatial frequency stimuli (i.e., fine detail). In the primate, this is generally not the case since spectrally opponent cells are found in the parvocelluar pathway which is sensitive to high spatial stimuli; nonopponent cells are found in the magnocelluar pathway which is not sensitive to high spatial stimuli. Therefore, lower vertebrate visual systems may not seem to be as complex compared to the primate, yet they function very well.

Through this study, the researcher has been able to identify the basic visual processes of zebrafish. It has been shown that zebrafish appear to have one visual channel and this channel seems to be responsible for processing chromatic information; therefore zebrafish do not process color and luminance information as separate phenomena. Evolutionary speaking, it is very interesting to speculate why the visual systems of higher vertebrates such as primates organize processing of color and luminance information differently than lower vertebrates. Separating chromatic and luminance processing into distinct anatomical pathways might serve as a survival mechanism for the primate. If one system is damaged, then the animal would still be able to have some visual capabilities.

Exactly why such an arrangement is found only in higher vertebrates is unclear, but could be related to a number of factors. For instance, higher vertebrates have more socially complex interactions and other behaviors. This organization of the visual system in higher vertebrates might be needed or at least more efficient for the environments in which higher vertebrates are found.

We assume that the organization of the primate visual system to be more efficient given it is most similar to human vision. But is it more efficient to keep color and luminance information separate? Many fish have been shown to have well developed color vision. As far as the zebrafish is concerned, having four cone types might indicate that enough information is extracted from the visual world without needing to maintain a segregation of luminance and color. Although this is speculation, it might be that color and luminance do not have to be processed separately, then recombined as done in higher vertebrates.

Future Directions

Future research related to topics addressed in this study should focus on a) further investigation on visual processing in zebrafish, and b) resolve some of the issues regarding parallel processing in lower vertebrates such as zebrafish. Of course focusing on the first issue will inevitably answer questions raised by the second. To extend the procedures and methodology used in this study, a number of changes could be made which would further address color vision capabilities in zebrafish. For example, it would be interesting to use an increment threshold technique with a monochromatic background. In this procedure, monochromatic stimuli of different intensities would be superimposed onto a monochromatic background of a fixed wavelength. The advantages of such a procedure are twofold: First, it would permit further insight to separate processing of color and luminance in zebrafish since chromatic adaptation has been shown to reveal color opponent mechanisms that normally are hidden (Mackintosh et al., 1987). Second, chromatic

adaptation has traditionally been a tool to determine cone contributions. The increment threshold procedure could be performed at four different spectral backgrounds, with each one designed to chromatically adapt one of the four cone types in zebrafish. Computational modeling could then be performed on the spectral sensitivity functions obtained under each chromatic background to help determine cone contributions.

The ERG provides only a gross measure of the electrical activity of the retina and cannot convey the response properties of single neurons. It would be interesting to examine single units in the zebrafish retina, classify these cells based on their spectral properties, and then conduct the increment threshold procedure to derive spectral sensitivity functions. Such a procedure would permit a direct comparison between ERG and single unit data in zebrafish. It has been stressed in this paper that further empirical studies are needed to assess retinal function in zebrafish.

Another aspect of visual processing that could corroborate the results obtained here is to determine how ganglion cells in the zebrafish retina process light increments and decrements. Separate visual channels for light increments and decrements are present in lower vertebrates such as the goldfish and have been found to contribute to color vision (see DeMarco & Powers, 1991). For example, DeMarco & Powers (1991) have shown that ON- and OFF-cells have different spectral sensitivities and receive a different combination of cone inputs. Furthermore, ON-and OFF-cells can be either spectrally opponent or nonopponent in goldfish (Bilotta & Abramov, 1989b). Examination of these cells in zebrafish would greatly add to knowledge about its visual capabilities.

The results of this thesis do have some applied value for those interested in research that might utilize the zebrafish as an animal model. The zebrafish has become a popular animal model in a number of scientific disciplines, including genetics and developmental biology. Some researchers in vision science have begun to use the zebrafish as an animal

model of vision. For example, genetics researchers are comparing mutants and normal young zebrafish in their visual sensitivity. However, this study is only one of two (see also Patterson, 1996) which have investigated visual processing in the adult zebrafish. Therefore, the results of this study contribute to a knowledge base about the capabilities of the zebrafish visual system. In addition, our laboratory has begun behavioral studies of the developing zebrafish visual system. In the near future, physiological studies will examine the spectral sensitivity of zebrafish at different ages. Therefore, future studies have the results of this thesis, which investigated visual function in the adult zebrafish, to compare with the developing zebrafish. Finally, much of the visual functioning in zebrafish remains to be studied. The zebrafish is a relatively new animal model so the avenues are clearly open for innovative research. Using this vertebrate model, many advances will certainly be made in understanding visual function.

References

Barinaga, M. (1990). Zebrafish swimming into the development mainstream. Science, 250, 34-35.

Bilotta, J., & Abramov, I. (1989a). Spatial properties of goldfish ganglion cells. Journal of General Physiology, 93, 1147-1169.

Bilotta, J., & Abramov, I. (1989b). Spatiospectral properties of goldfish ganglion cells. Journal of Neurophysiology, 62, 1140-1148.

Bilotta, J., Dobis, W. T., Googe, M. D., Nunley, W. R., & DeLorenzo, A. S. (1996). The effects of abnormal lighting conditions on zebrafish visual development. Investigative Ophthalmology and Visual Science Supplement, 37, 631.

Boynton, R. M. (1979). Human Color Vision. New York: Holt, Rinehart, and Winston.

Branchek, T. (1984). The development of photoreceptors in the zebrafish, Brachydanio rerio. II. Function. Journal Comparative Neurology, 224, 116-122.

Cornsweet, T. N. (1977). Visual Perception. New York: Academic Press.

Coughlin, D. J., & Hawryshyn, C. W. (1994a). The contribution of ultraviolet and short-wavelength sensitive cone mechanisms to color vision in rainbow trout. Brain Behavior and Evolution, 43, 219-232.

Coughlin, D. J., & Hawryshyn, C. W. (1994b). Ultraviolet sensitivity in the torus semicircularis of juvenile rainbow trout (*Oncorhynchus mykiss*). Vision Research, 34, 1407-1413.

DeMarco, P. J., & Powers, M. K. (1989). Sensitivity of ERG components from dark-adapted goldfish retinas treated with APB. Brain Research, 482, 317-323.

DeMarco, P. J., & Powers, M. K. (1991). Spectral sensitivity of ON and OFF responses from the optic nerve of goldfish. Visual Neuroscience, 6, 207-217.

DeValois, R. L., Abramov, I., & Jacobs, G. (1966). Analysis of response patterns of LGN cells. Journal of the Optical Society of America, 56, 966-977.

Eisner, A., & Macleod, D. A. (1979). Blue-sensitive cones do not contribute to luminance. Journal of the Optical Society of America, 70, 121-123.

Flamarique, I. N., & Hawryshyn, C. W. (1996). Retinal development and visual sensitivity of young pacific sockeye salmon (*Oncorhynchus nerka*). Journal of Experimental Biology, 199, 869-882.

Gates, S. C., & Becker, J. (1989). Laboratory Automation Using the IBM-PC. New Jersey: Princeton Hall.

Goldsmith, T. H. (1994). Ultraviolet receptors and color vision: Evolutionary implications and a dissonance of paradigms. Vision Research, 34, 1479-1487.

Gouras, P., & Zrenner, E. (1979). Enhancement of luminance flicker by color-opponent mechanisms. Science, 205, 587-589.

Gouras, P., & Zrenner, E. (1981). Color coding in the primate retina. Vision Research, 21, 1591-1598.

Hawryshyn, C. W., & Beauchamp, R. D. (1985). Ultraviolet photosensitivity in goldfish: An independent u.v. retinal mechanism. Vision Research, 25, 11-20.

Hawryshyn, C. W., Chou, B. R., & Beauchamp, R. D. (1985). Ultraviolet transmission by the ocular media of goldfish: Implications for ultraviolet photosensivity in fishes. Canadian Journal of Zoology, 63, 1244-1251.

Hawryshyn, C. W., & Harosi, F. I. (1991). Ultraviolet photoreception in carp: Microspectrophotometry and behaviorally determined action apectra. Vision Research, 31, 567-576.

Hawryshyn, C. W., & Harosi, F. I. (1994). Spectral characteristics of visual pigments in rainbow trout (*Oncorhynchus mykiss*). Vision Research, 34, 1385-1392.

Jacobs, G. H., & Deegan, J. F. (1994). Sensitivity to ultraviolet light in the gerbil (*Meriones unguiculatus*): Characteristics and mechanisms. Vision Research, 34, 1433-1441.

Keppel, G. (1991). Design and Analysis (3rd ed.). Englewood Cliffs, NJ: Princeton Hall.

King-Smith, P. E., & Carden, D. (1976). Luminance and opponent color contributions to visual detection and adaptation to temporal and spatial integration. Journal of the Optical Society of America, 66, 709-717.

Knowles, A., & Dartnall, H. J. A. (1977). Spectroscopy of visual pigments. In H. Davson (Ed.), The Eye (Vol. 2B, pp. 74-84). New York: Academic Press.

Kuffler, S. W. (1953). Discharge patterns and functional organization of the mammalian retina. Journal of Neurophysiology, 6, 37-68.

Laale, H. W. (1977). Biology and use of zebrafish, *Brachiodanio rerio,* in fisheries research. Journal of Fish Biology, 10, 121-173.

Lennie, P. (1980). Parallel pathways in the visual system. Vision Research, 20, 561-594.

Levine, M. W., & Shefner, J. M. (1981). Fundamentals of Sensation and Perception. Reading, MA: Addison-Wesley Publishing Company.

Livingstone, M. S., & Hubel, D. H. (1987). Psychophysical evidence for separate channels for the perception of form, color, movement, and depth. Journal of Neuroscience, 7, 3416-3468

Livingstone, M. S., & Hubel, D. H. (1988). Segregation of form, color, movement, and depth: Anatomy, physiology, and perception. Science, 240, 740-749.

Loop, M. S., Millican C. L., & Thomas, S. R. (1987). Photopic spectral sensitivity of the cat. Journal of Physiology, 382, 537-553.

Mackintosh, R. M., Bilotta, J., & Abramov, I. (1987). Contributions of short-wavelength cones to goldfish ganglion cells. Journal of Comparative Physiology A, 161, 85-94.

Merigan, W. H. (1989). Chromatic and achromatic vision of macaques: Role of the p pathway. Journal of Neuroscience, 9, 776-783.

Merigan, W. H., & Maunsell, J H. R. (1990). Macaque vision after magnocellular lateral geniculate lesions. Visual Neuroscience, 5, 347-352.

Merigan, W. H., & Maunsell, J. H. R. (1993). How parallel are the primate visual pathways? Annual Review of Neuroscience, 16, 369-416.

Mills, S. L., & Sperling, H. G. (1990). Red/green opponency in the rhesus macaque ERG: Spectral sensitivity is reduced by bicuculline. Visual Neuroscience, 5, 217-221.

Nawrocki, L., Bremiller, R., Streisinger, G., & Kaplan, M. (1985). Larval and adult visual pigments of the zebrafish, *Brachydanio rerio*. Vision Research, 25, 1569-1576.

Neumeyer, C. (1984). On spectral sensitivity in the goldfish. Vision Research, 24, 1223-1231.

Neumeyer, C. (1985). An ultra-violet receptor as a fourth receptor type in goldfish color vision. Naturwissenschaften, 72, 162-163.

Neumeyer, C., Wietsma, J. J., & Spekreijse, H. (1991). Separate processing of "color" and "brightness" in goldfish. Vision Research, 31, 537-549.

Nicole, J. A. C. (1989). The Eyes of Fishes. Oxford: Clarendon Press

Patterson, W. F. II. (1996). Temporal characteristics of zebrafish cone types and their role in separate visual processing. Unpublished master's thesis, Western Kentucky University.

Petry, H. M. (1993). Long-term changes in visual mechanisms following differential stimulation of color and luminance channels during development. In T. P. HIcks, S. Molorchnikoff, & T. Ono (Eds.), Progress in Brain Research (pp. 235-250). San Diego, CA: Academic Press.

Petry, H. M., & Kelly, J. P. (1991). Psychophysical measurement of spectral sensitivity and color vision in red-light-reared tree shrews (*Tupaia Belangeri*). Vision Research, 31, 1749-1757.

Regan, D., Schellart, N. A. M., Spekreijse, H., & van den Berg, T. J. T. P. (1975). Photometry of goldfish by electrophysiological recording: Comparison of criterion response method with heterochromatic flicker photometry. Vision Research, 15, 799-807.

Robinson, J., Schmitt, E. A., Harosi, F. I., Reece, R. J., & Dowling, J. E. (1993). Zebrafish ultraviolet visual pigment: Absorption spectrum, sequence, and localization. Proceedings of the National Academy of Science USA, 90, 6009-6012.

Schiller, P. H., & Logothetis, N. K. (1990). The color-opponent and broad-band channels of the primate visual system. Trends in Neuroscience, 13, 392-398.

Schiller, P. H., Logothetis, N. K., & Charles, E. R. (1990). Role of the color-opponent and broad-band channels in vision. Visual Neuroscience, 5, 321-346.

Schiller, P. H., Logothetis, N. K., & Charles, E. R. (1991). Parallel pathways in the visual system: Their role in the perception at isoluminance. Neuropsychologia, 29, 433-441.

Schiller, P. H., & Malpeli, J. G. (1978). Functional specificity of lateral geniculate nucleus laminae of the rhesus monkey. Journal of Neurophysiology, 41, 788-797.

Sekuler, R., & Blake, R. (1994). Perception (3rd ed.). New York: McGraw-Hill.

Shapley, R. (1990). Visual sensitivity and parallel retinocortical channels. Annual Review of Psychology, 41, 635-658.

Spekreijse, H., Wietsma, J. J., & Neumeyer, C. (1991). Induced color blindness in goldfish: A behavioral and electrophysiological study. Vision Research, 31, 551-562.

Sperling, H. G., & Harwerth, R. S. (1971). Red-green cone interactions in the increment threshold spectral sensitivity of primates. Science, 172, 180-184.

Vennat, J. C., Besse, G., Sanzelle, S., Doly, M., & Gaillard, G. (1994). A program for automatic acquisition and process of electroretinograms in vivo in albino rats. Journal of Neuroscience Methods, 52, 17-21.

Yager, D. (1967). Behavioral measures and theoretical analysis of spectral sensitivity and spectral saturation in the goldfish. Vision Research, 7, 707-727.

Zrenner, E., Abramov, I., Akita, M., Cowey, A., Livingstone, M., & Valberg, A. (1990). Color perception: Retina to cortex. In L. Spillmann, & J. S. Werner (Eds.), Visual Perception: The Neurophysiological Foundations (pp.163-204). San Diego, CA: Academic Press.

Appendix A

Irradiance at 0.0 Log Units Attenuation at Each Stimulus Wavelength

Wavelength (nanometers)	Irradiance (quanta/cm^2/s)
320	2.14×10^{11}
340	5.81×10^{11}
360	5.67×10^{11}
380	1.21×10^{12}
400	2.90×10^{12}
420	6.49×10^{12}
440	7.80×10^{12}
480	1.05×10^{13}
520	1.04×10^{13}
560	1.12×10^{13}
600	8.85×10^{12}
640	1.02×10^{13}
660	1.02×10^{13}

Table 1.

Relative Weights of the Four Cone Types in Zebrafish Based on Spectral Sensitivity Functions Derived Under High and Low Intensity Background Adaptation.

	Background Adaptation									
	Low Background Intensity					High Background Intensity				
Subject	U	S	M	L	SS	U	S	M	L	SS
AP	.58	.05	.23	.11	.51	.74	.09	.09	.01	.16
AQ	.58	.00	.60	.25	.71	.61	.34	.17	.05	.22
AR	.59	-.12	.09	-.01	.38	.96	.01	.11	.01	.05
AS	.60	-.08	.50	.10	.57	.65	.21	.17	.02	.33
AU	.77	-.24	.46	.21	.44	.66	-.01	.12	.00	.26
AV	.02	-.32	.74	-.10	.29	.68	.27	.06	.04	.28
AW	.54	.14	.74	.18	.36	.86	.04	.10	.02	.29
AY	.69	-.23	.72	.27	.31	.84	-.02	.08	.00	.13
AZ	.38	-.30	.84	.33	.21	.90	-.03	.11	.03	.22
BA	.56	-.18	.71	.20	.32	.94	-.04	.12	.02	.19
Mean	.64	-.23	.74	.13	.25	.95	.09	.14	.02	.14

Note. U=ultraviolet cones, S=short cones, M= middle cones, L=Long cones, and SS=sum of squares. Mean represents the relative weights of each cone type to the average spectral sensitivity function from ten fish.

Figure Captions

Figure 1. Zebrafish electroretinograms (ERGs) obtained with a 400 nm stimulus at -2.0 log units attenuation under -1.0 log unit white background (4.36 x 10^2 mW/cm^2). With no attenuation, the irradiance of the 400 nm stimulus was 2.90 x 10^{12} quanta/cm^2/s. Figure 1a shows the unfiltered ERGs and Figure 1b shows the same ERGs after they were filtered for 60 Hz noise. The raised bars in each figure mark the onset and termination of the test stimulus.

Figure 2. Figure 2a shows an averaged ERG obtained with a 400 nm stimulus at 2.0 log units attenuation under -1.0 log unit white background. With no attenuation, the irradiance of the 400 nm stimulus was 2.90 x 10^{12} quanta/cm^2/s. This is the same ERG response as in Figure 1b after the three responses were averaged. Figure 2b shows averaged ERGs at different stimulus irradiances. Each of the averaged ERGs shown in Figure 2b was obtained with a 400 nm stimulus under -1.0 log unit white background. The ERG with the -2.0 log unit attenuation is the same ERG that is shown in Figure 2a. The other ERGs were obtained with stimulus attenuations of -2.5, -3.0, and -4.0 log units. For all averaged ERG's the baseline responses were set to zero microvolts. See text for details.

Figure 3. Irradiance-response functions obtained with 400 nm (Figure 3a) and 600 nm (Figure 3b) stimuli under -1.0 log unit white background. The irradiance for the 400 nm stimulus at 0.0 attenuation was 2.90 x 10^{12} quanta/cm^2/s; the irradiance for the 600 nm stimulus was 8.85 x 10^{12} quanta/cm^2/s.

Figure 4 Spectral sensitivity function for one fish derived under -1.0 log unit white background adaptation. Sensitivity was determined by finding the stimulus irradiance that yielded a criterion response of +50 microvolts.

Figure 5. Average spectral sensitivity functions derived under -1.0 log (squares) and -6.0 log unit white background (triangles). Sensitivity was determined by finding the stimulus irradiance that yielded a criterion response of +50 microvolts. Error bars represent ±1 SEM.

Figure 6. Spectral sensitivity functions constructed from test sessions in which stimulus wavelength presentation began either at 320 nm (triangles) or 660 nm (squares). Each spectral sensitivity function in Figures 6a and 6b is based on five test sessions. Figure 6a shows spectral sensitivity functions derived under -6.0 log unit white background and Figure 6b shows spectral sensitivity functions derived under -1.0 log unit white background. Sensitivity was determined by finding the stimulus irradiance that yielded a criterion response of +50 microvolts. Error bars represent ±1 SEM.

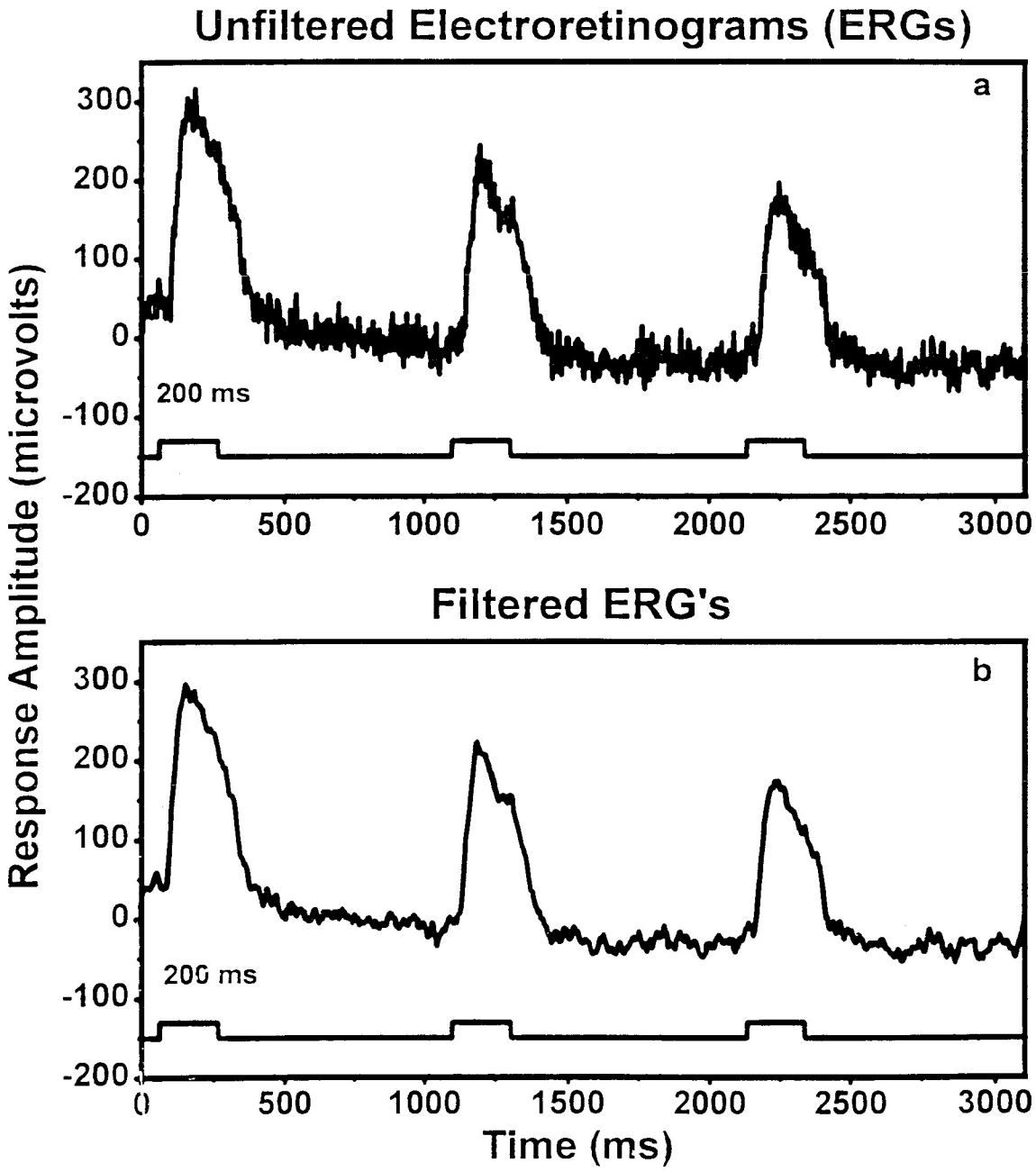
Figure 7. Spectral sensitivity functions based on test sessions in which fish were adapted first to either the -6.0 log unit white background or the -1.0 log unit white background. Figure 7a shows spectral sensitivity functions derived under -6.0 log unit white background. The spectral sensitivity function with triangles represents data from seven fish first adapted to the -6.0 log unit white background and the spectral sensitivity function with squares represents data from three fish first adapted to the -1.0 log unit white background. Figure 7b shows spectral sensitivity functions derived under -1.0 log unit white background. The spectral sensitivity function with triangles represents data from seven fish first adapted to the -6.0 log unit white background and the spectral sensitivity function with squares represents data from three fish first adapted to the -1.0 log unit white background. Sensitivity was determined by finding the stimulus irradiance that yielded a criterion response of +50 microvolts. Error bars represent ±1 SEM.

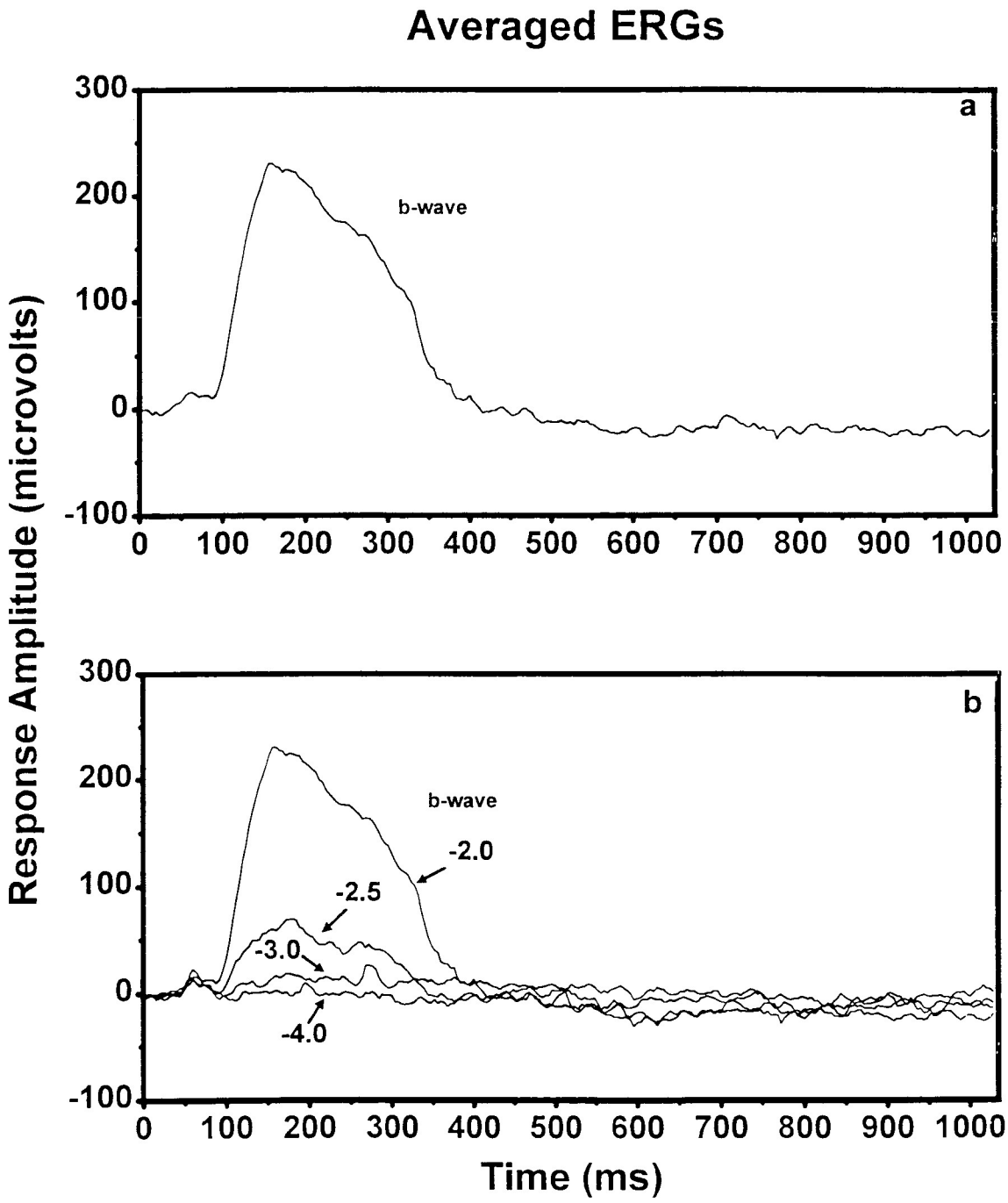
Figure 8. Cone absorptance spectra based on rhodopsion for the four cone types in zebrafish. 0.0 log relative absorptance = 100% absorptance. Solid line = U-cones; dashed line = S-cones; dotted dashed line = M-cones; double dotted dashed line = L-cones. The λ-max values for the U-, S-, M-, and L-cones are 360, 420, 480, and 560 nm, respectively. See text for details.

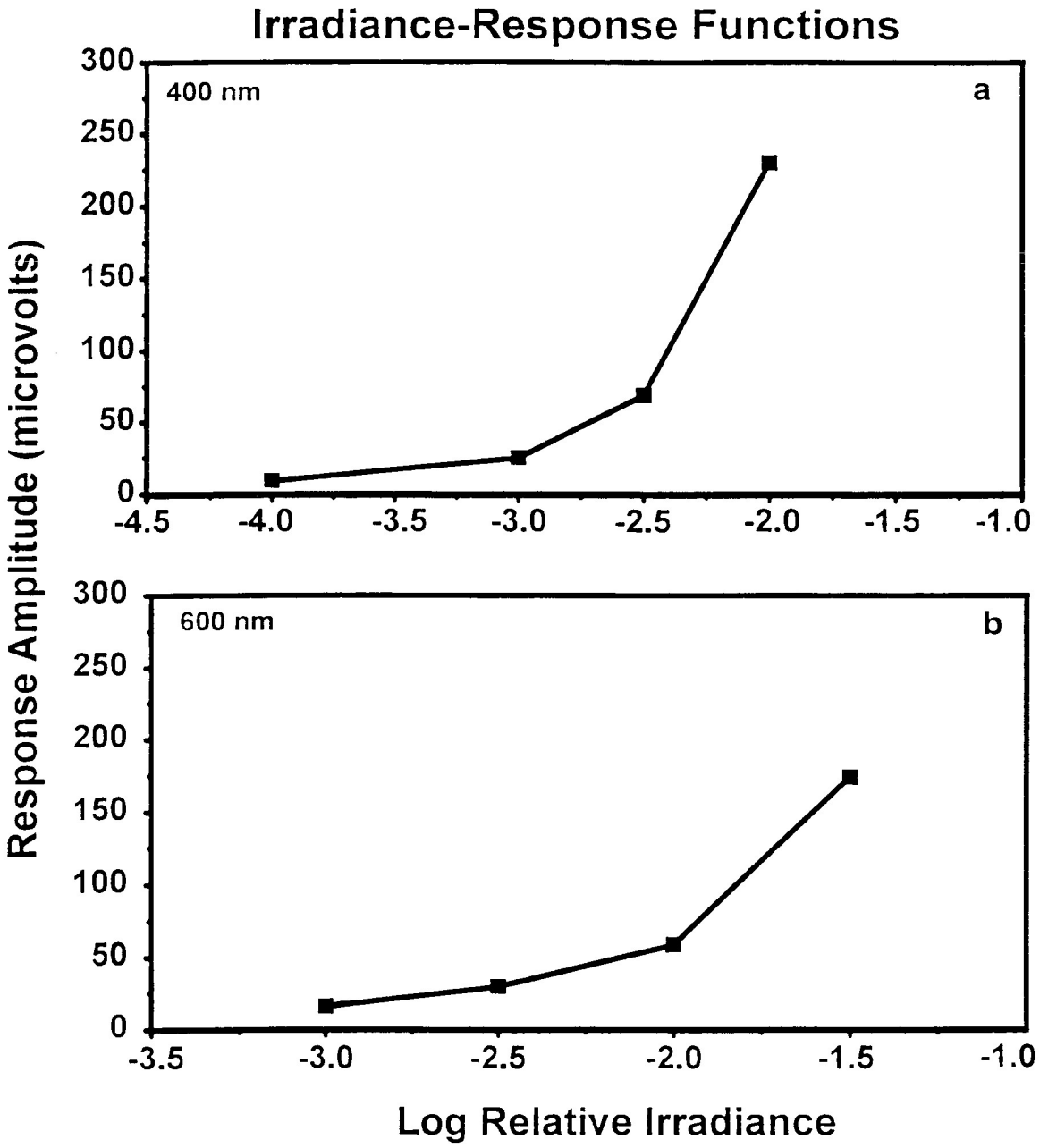
Figure 9. Spectral sensitivity function showing the normalized average spectral sensitivity function (squares) of ten fish under the -1.0 log unit white background. The best fitting curve (solid line) from the linear model is given along with model weights.

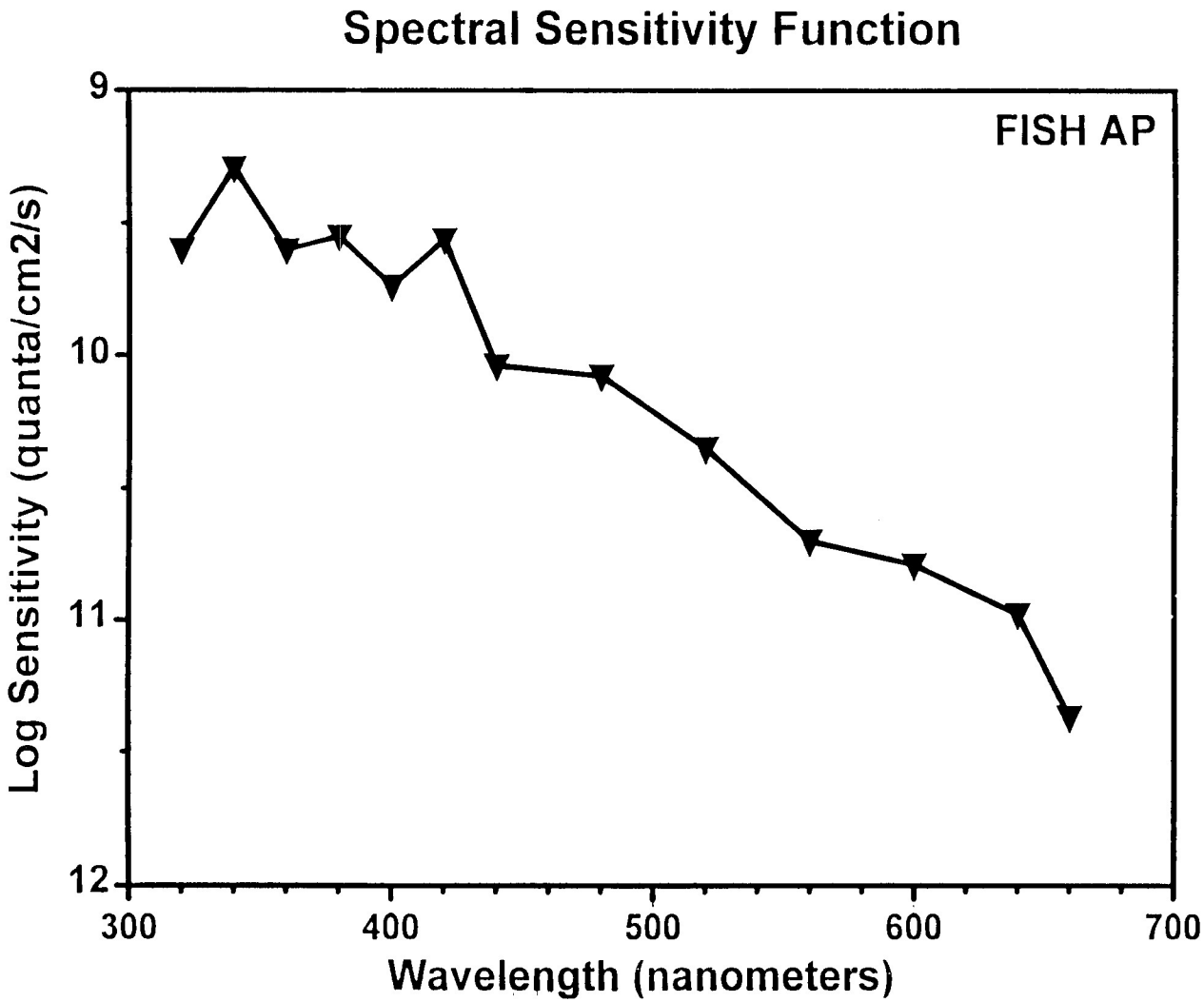
Figure 10. Spectral sensitivity function showing the normalized average spectral sensitivity function (squares) of ten fish under the -6.0 log unit white background. The best fitting curve (solid line) from the linear model is given along with model weights.

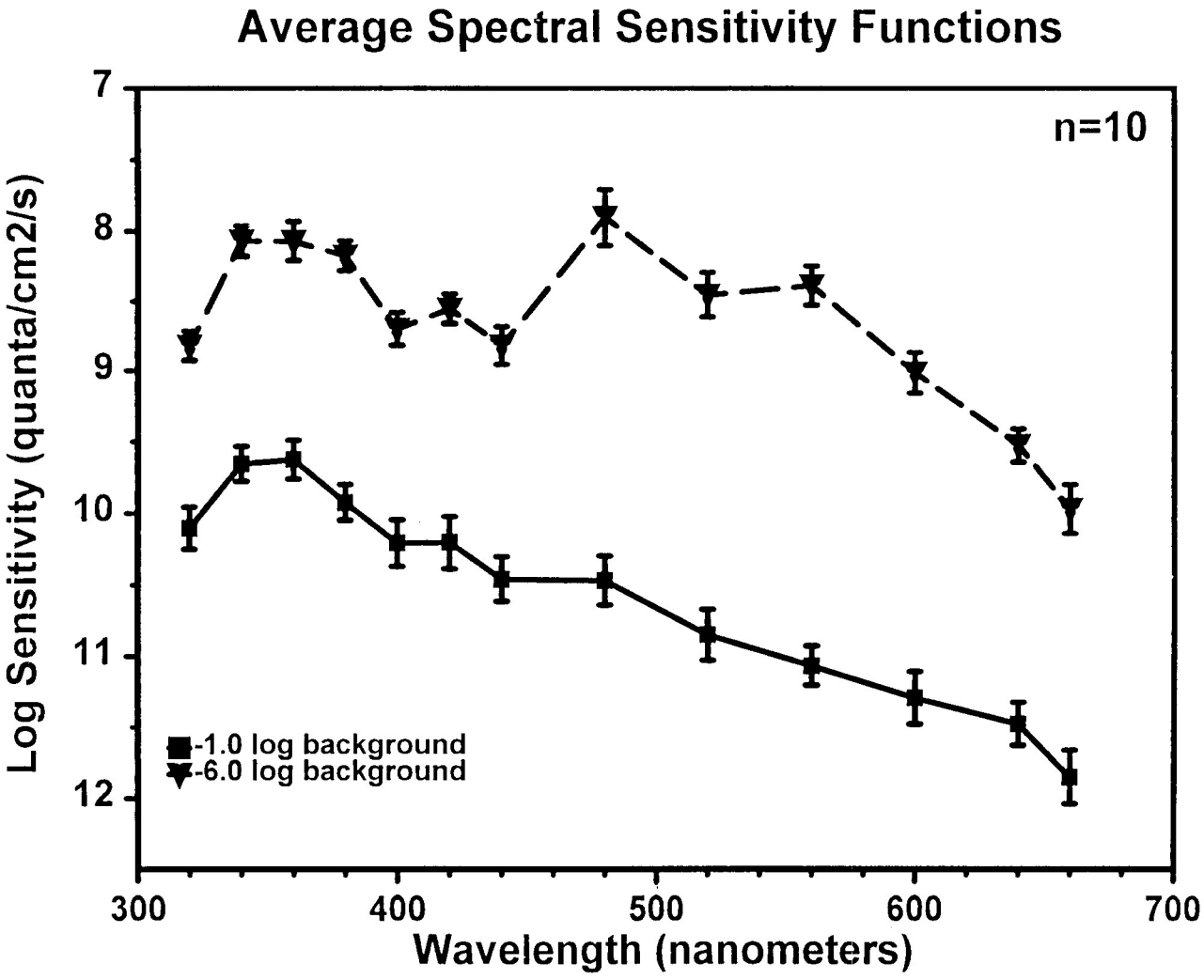
Figure 11. Mean relative cone weights from the linear model derived from the spectral sensitivity functions of ten fish. Relative cone weights for -1.0 log unit and -6.0 log unit white background conditions are shown.

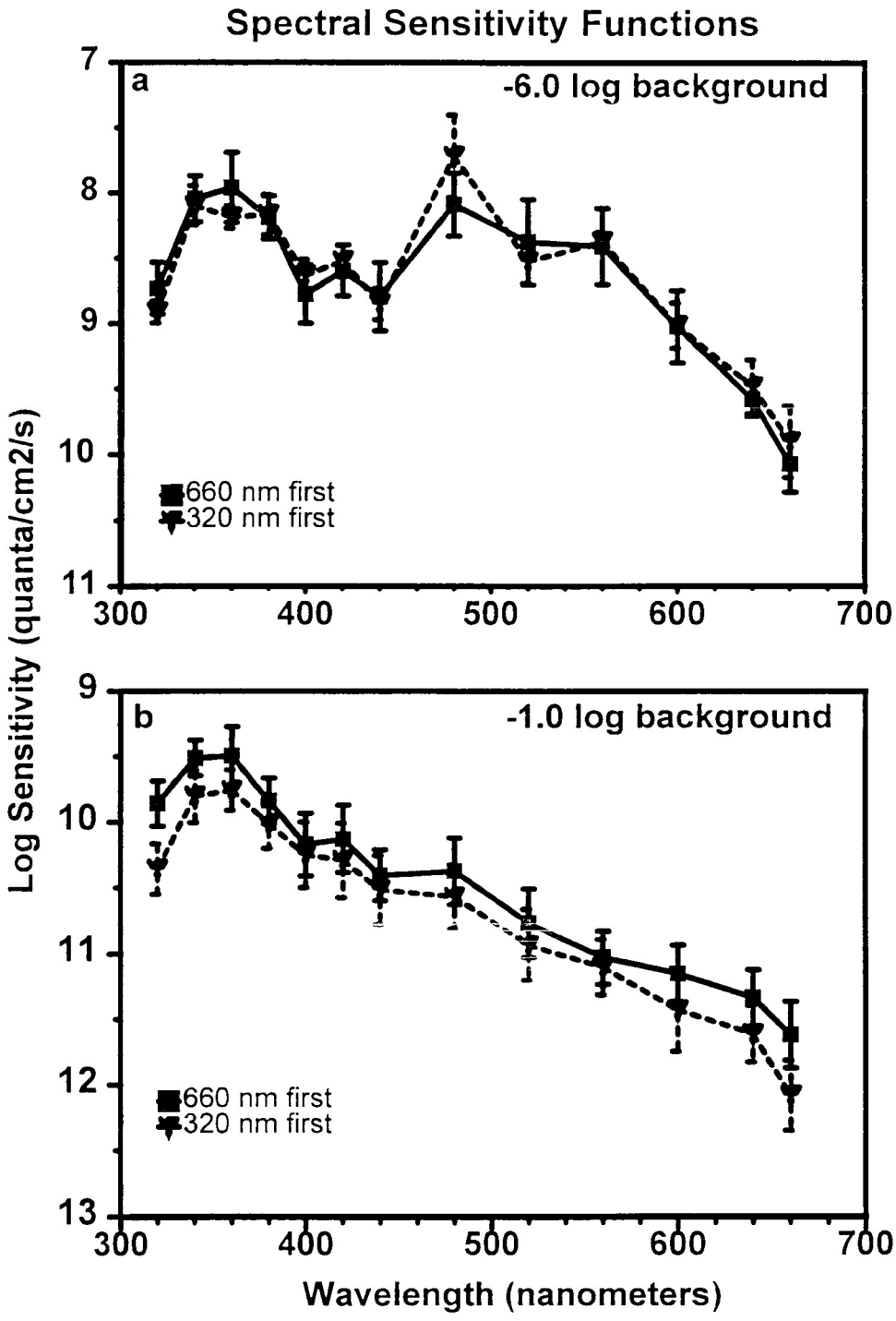

Unfiltered Electroretinograms (ERGs)
a
300
200
100
0
-100
-200
200 ms
0
500
1000
1500
2000
2500
3000
Filtered ERG's
b
300
200
100
0
-100
-200
200 ms
0
500
1000
1500
2000
2500
3000
Response Amplitude (microvolts)
Time (ms)

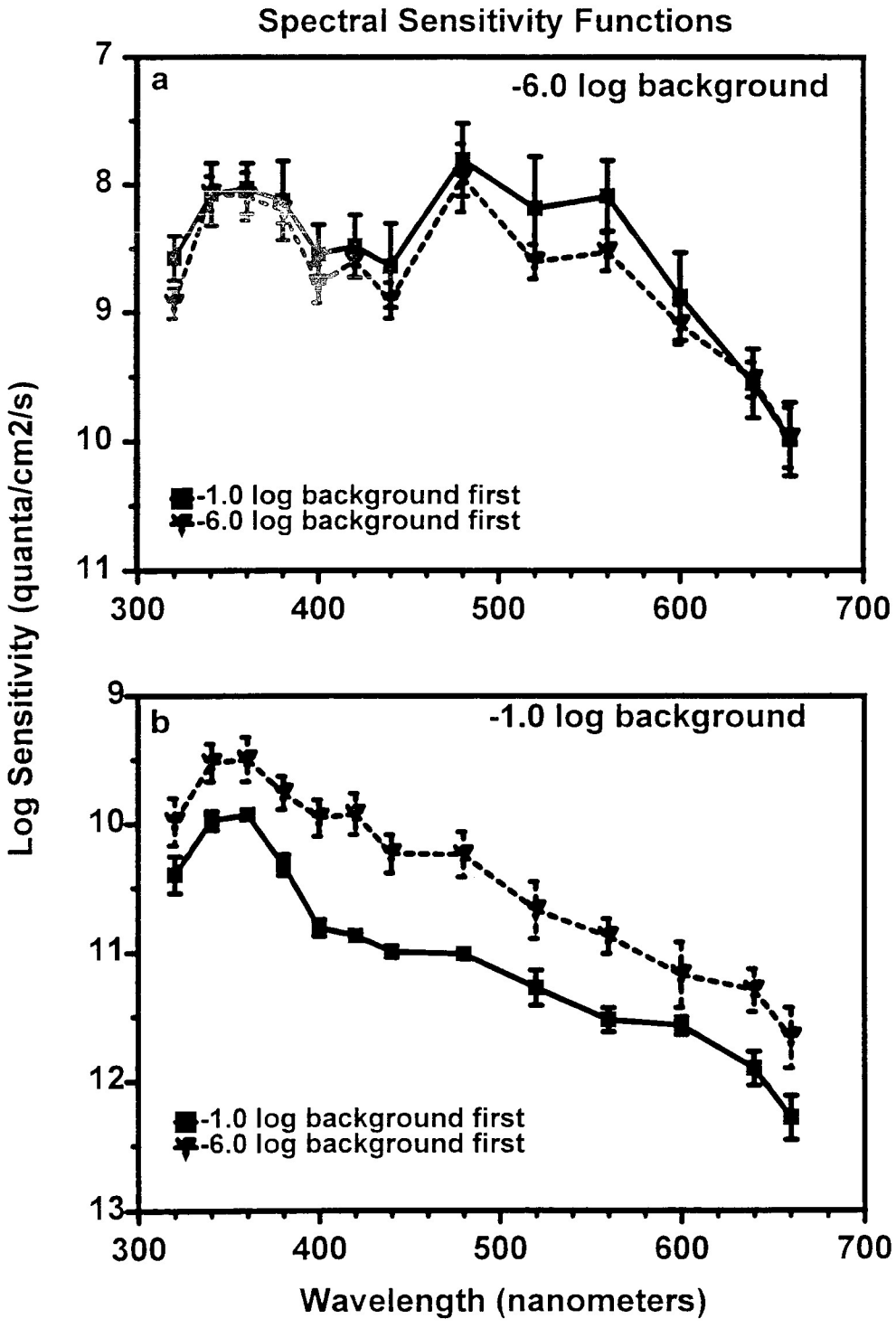

Averaged ERGs
a
b-wave
b
b-wave
-2.0
-2.5
-3.0
-4.0
Response Amplitude (microvolts)
Time (ms)
300
200
100
0
-100
0
100
200
300
400
500
600
700
800
900
1000

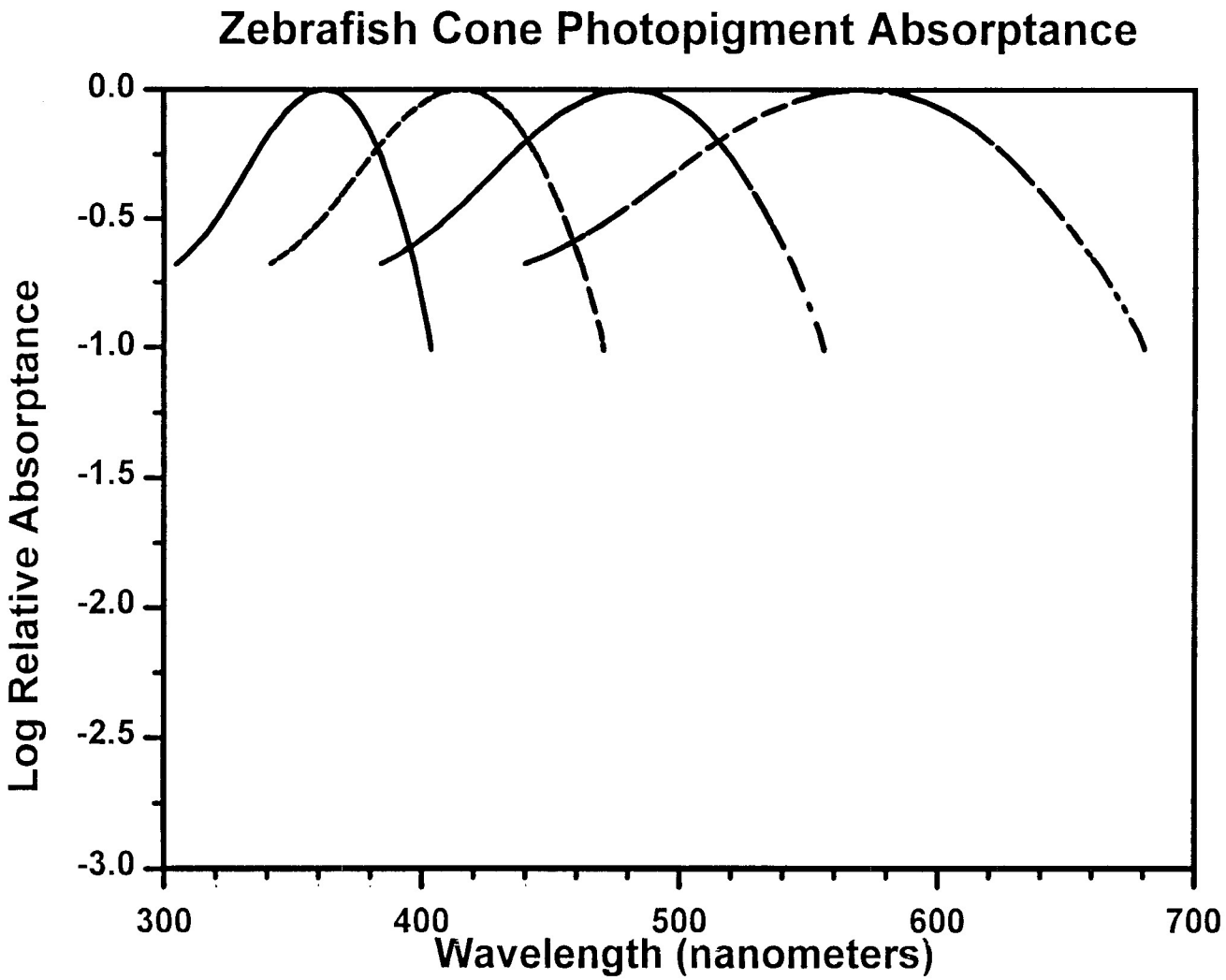

Irradiance-Response Functions
400 nm
a
600 nm
b
Response Amplitude (microvolts)
Log Relative Irradiance
300
250
200
150
100
50
0
-4.5
-4.0
-3.5
-3.0
-2.5
-2.0
-1.5
-1.0

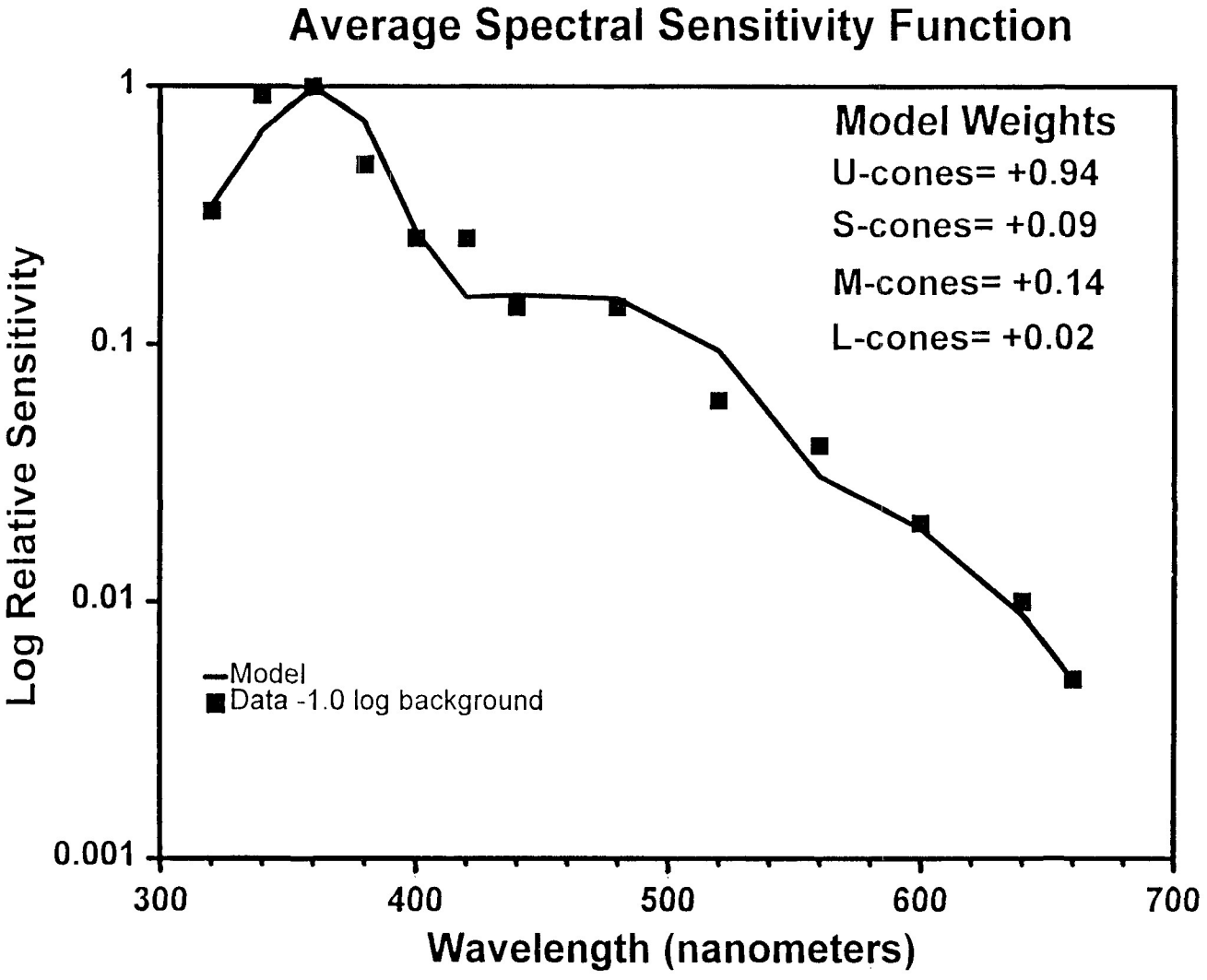

Spectral Sensitivity Function
FISH AP
Log Sensitivity (quanta/cm2/s)
9
10
11
12
300
400
500
600
700
Wavelength (nanometers)

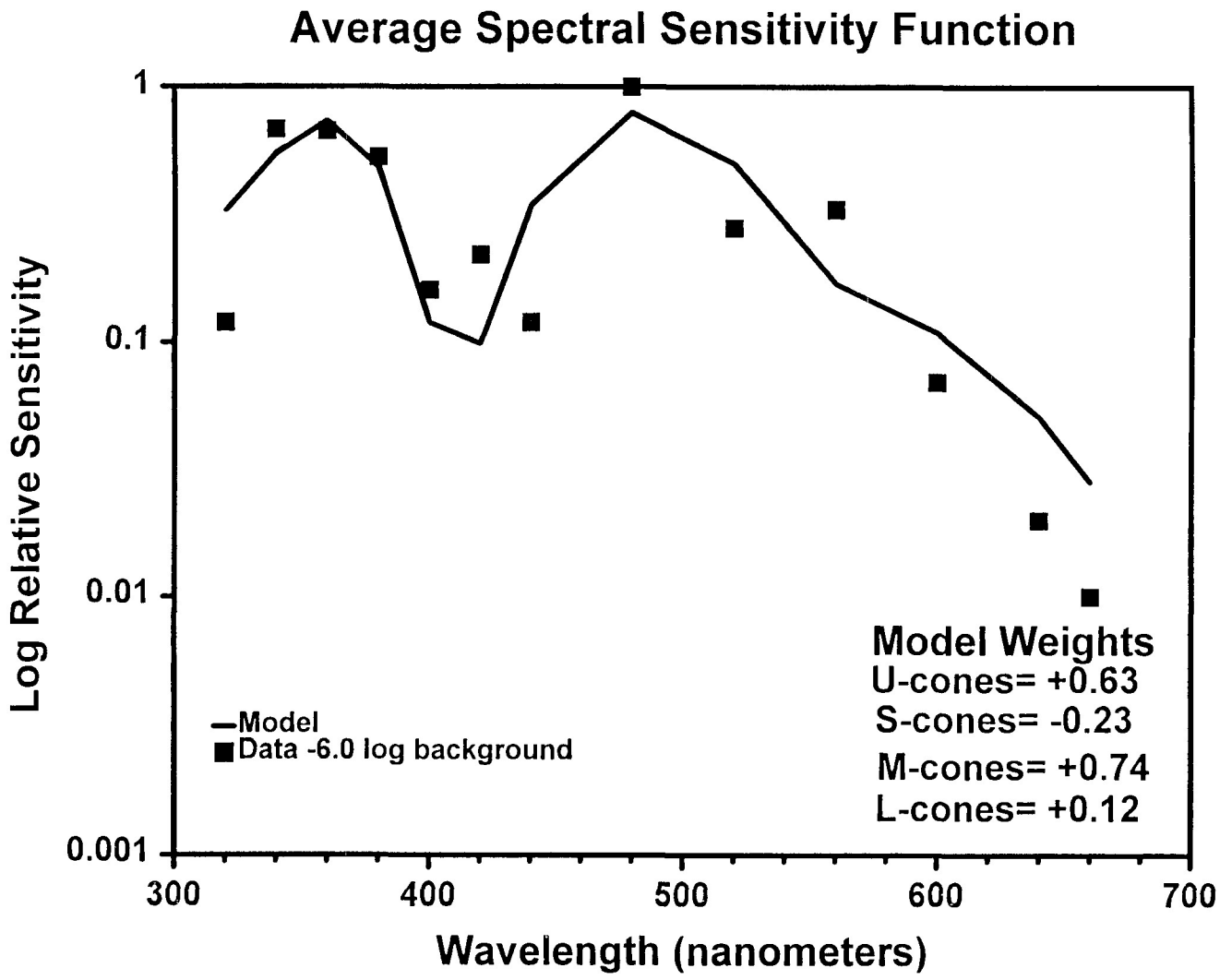

Average Spectral Sensitivity Functions
n=10
Log Sensitivity (quanta/cm2/s)
7
8
9
10
11
12
300
400
500
600
700
Wavelength (nanometers)
-1.0 log background
-6.0 log background

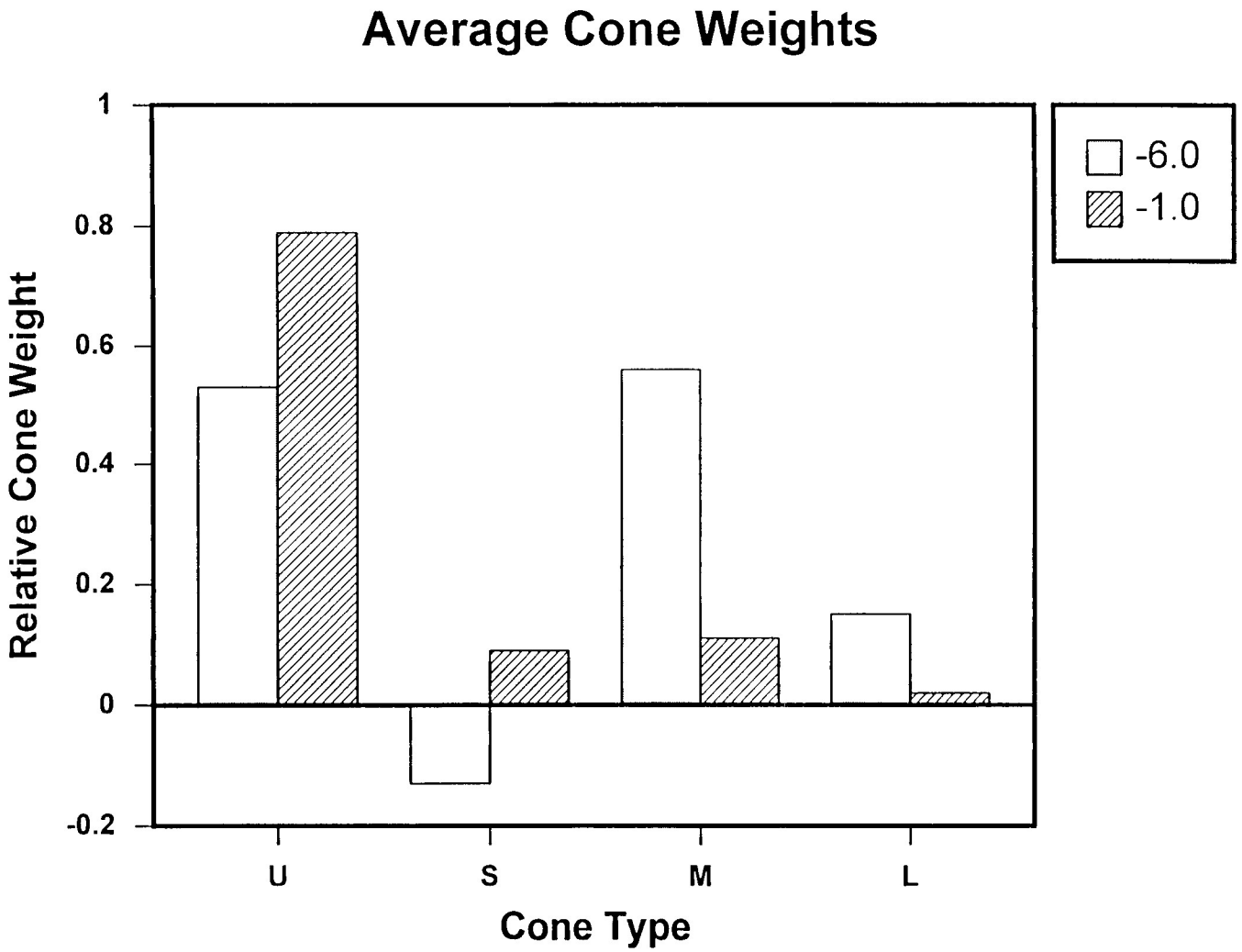

Spectral Sensitivity Functions
a
-6.0 log background
660 nm first
320 nm first
b
-1.0 log background
660 nm first
320 nm first
Log Sensitivity (quanta/cm2/s)
Wavelength (nanometers)
7
8
9
10
11
12
13
300
400
500
600
700


Spectral Sensitivity Functions
a
-6.0 log background
-1.0 log background first
-6.0 log background first
b
-1.0 log background
-1.0 log background first
-6.0 log background first
Log Sensitivity (quanta/cm2/s)
Wavelength (nanometers)
7
8
9
10
11
12
13
300
400
500
600
700


Zebrafish Cone Photopigment Absorptance
Log Relative Absorptance
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
300
400
500
600
700
Wavelength (nanometers)


Average Spectral Sensitivity Function
Model Weights
U-cones= +0.94
S-cones= +0.09
M-cones= +0.14
L-cones= +0.02
Model
Data -1.0 log background
Log Relative Sensitivity
1
0.1
0.01
0.001
300
400
500
600
700
Wavelength (nanometers)


Average Spectral Sensitivity Function
Log Relative Sensitivity
1
0.1
0.01
0.001
300
400
500
600
700
Wavelength (nanometers)
—Model
■Data -6.0 log background
Model Weights
U-cones= +0.63
S-cones= -0.23
M-cones= +0.74
L-cones= +0.12


Average Cone Weights
Relative Cone Weight
1
0.8
0.6
0.4
0.2
0
-0.2
U
S
M
L
Cone Type
-6.0
-1.0